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(54) Title: GENES OF CAROTENOID BIOSYNTHESIS AND METABOLISM AND METHODS OF USE THEREOF

(57) Abstract

Nucleic acid sequences encoding  $\epsilon$ -cyclase, isopentenyl pyrophosphate isomerase and  $\beta$ -carotene hydroxylase as well as vectors containing the same and hosts transformed with the vectors. Methods for controlling the ratio of various carotenoids in a host and for the production of novel carotenoid pigments. The present invention also provides a method for screening for eukaryotic genes encoding carotenoid biosynthesis, and for modifying the disclosed enzymes.

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# GENES OF CAROTENOID BIOSYNTHESIS AND METABOLISM AND METHODS OF USE THEREOF

## BACKGROUND OF THE INVENTION

#### Field of the Invention

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The present invention describes nucleic acid sequences for eukaryotic genes encoding  $\epsilon$  lycopene  $\epsilon$ -cyclase (also known as  $\epsilon$ -cyclase and  $\epsilon$  lycopene cyclase), isopentenyl pyrophosphate isomerase (IPP) and  $\beta$ -carotene hydroxylase as well as vectors containing the same and hosts transformed with said vectors. The present invention also provides methods for augmenting the accumulation of carotenoids, changing the composition of the carotenoids, and producing novel and rare carotenoids. The present invention provides methods for controlling the ratio or relative amounts of various carotenoids in a host. The invention also relates to modified lycopene  $\epsilon$ -cyclase, IPP isomerase and  $\beta$ -carotene hydroxylase. Additionally, the present invention provides a method for screening for genes and cDNAs encoding enzymes of carotenoid biosynthesis and metabolism.

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#### Background of the Invention

Carotenoid pigments with cyclic endgroups are essential components of the photosynthetic apparatus in oxygenic photosynthetic organisms (e.g., cyanobacteria, algae and plants; Goodwin, 1980). The symmetrical bicyclic yellow carotenoid pigment βcarotene (or, in rare cases, the asymmetrical bicyclic  $\alpha$ -carotene) is intimately associated with the photosynthetic reaction centers and plays a vital role in protecting against potentially lethal photooxidative damage (Koyama, 1991). β-carotene and other carotenoids derived from it or from α-carotene also serve as light-harvesting pigments (Siefermann-Harms, 1987), are involved in the thermal dissipation of excess light energy captured by the lightharvesting antenna (Demmig-Adams & Adams, 1992), provide substrate for the biosynthesis of the plant growth regulator abscisic acid (Rock & Zeevaart, 1991; Parry & Horgan, 1991), and are precursors of vitamin A in human and animal diets (Krinsky, 1987). Plants also exploit carotenoids as coloring agents in flowers and fruits to attract pollinators and agents of seed dispersal (Goodwin, 1980). The color provided by carotenoids is also of agronomic value in a number of important crops. Carotenoids are currently harvested from a variety of organisms, including plants, algae, yeasts, cyanobacteria and bacteria, for use as pigments in food and feed.

The probable pathway for formation of cyclic carotenoids in plants, algae and cyanobacteria is illustrated in Figure 1. Two types of cyclic endgroups or rings are commonly found in higher plant carotenoids, these are referred to as the  $\beta$  (beta) and  $\epsilon$  (epsilon) rings (Fig. 3). The precursor acyclic endgroup (no ring structure) is referred to as the  $\Psi$  (psi) endgroup. The  $\beta$  and  $\epsilon$  endgroups differ only in the position of the double bond in the ring. Carotenoids with two  $\beta$  rings are ubiquitous, and those with one  $\beta$  and one  $\epsilon$  ring are common, but carotenoids with two  $\epsilon$  rings are uncommon.  $\beta$ -carotene (Fig. 1) has two  $\beta$ -endgroups and is a symmetrical compound that is the precursor of a number of other important plant carotenoids such as zeaxanthin and violaxanthin (Fig. 2).

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Genes encoding enzymes of carotenoid biosynthesis have previously been isolated from a variety of sources including bacteria (Armstrong et al., 1989, Mol. Gen. Genet. 216, 254-268; Misawa et al., 1990, J. Bacteriol., 172, 6704-12), fungi (Schmidhauser et al., 1990, Mol. Cell. Biol. 10, 5064-70), cyanobacteria (Chamovitz et al., 1990, Z. Naturforsch, 45c, 482-86; Cunningham et al., 1994) and higher plants (Bartley et al., Proc. Natl. Acad. Sci USA 88, 6532-36; Martinez-Ferez & Vioque, 1992, Plant Mol. Biol. 18, 981-83). Many of the isolated enzymes show a great diversity in structure, function and inhibitory properties between sources. For example, phytoene desaturases from the cyanobacterium Synechococcus and from higher plants and green algae carry out a two-step desaturation to yield  $\zeta$ -carotene as a reaction product. In plants and cyanobacteria a second enzyme ( $\zeta$ carotene desaturase), similar in amino acid sequence to the phytoene desaturase, catalyzes two additional desaturations to yield lycopene. In contrast, a single desaturase enzyme from Erwinia herbicola and from other bacteria introduces all four double bonds required to form lycopene. The Erwinia and other bacterial desaturases bear little amino acid sequence similarity to the plant and cyanobacterial desaturase enzymes, and are thought to be of unrelated ancestry. Therefore, even with a gene in hand from one source, it may be difficult to identify a gene encoding an enzyme of similar function in another organism. In particular, the sequence similarity between certain of the prokaryotic and eukaryotic genes encoding enzymes of carotenoid biosynthesis is quite low.

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Further, the mechanism of gene expression in prokaryotes and eukaryotes appears to differ sufficiently such that one cannot expect that an isolated eukaryotic gene will be properly expressed in a prokaryotic host.

The difficulties in isolating genes encoding enzymes with similar functions is exemplified by recent efforts to isolate the gene encoding the enzyme that catalyzes the formation of  $\beta$ -carotene from the acyclic precursor lycopene. Although a gene encoding an enzyme with this function had been isolated from a bacterium, it had not been isolated from any photosynthetic procaryote or from any eukaryotic organism. The isolation and characterization of the enzyme catalyzing formation of  $\beta$ -carotene in the cyanobacterium Synechococcus PCC7942 was described by the present inventors and others (Cunningham et al., 1993 and 1994). The amino acid sequence similarity of the cyanobacterial enzyme to the various bacterial lycopene  $\beta$ -cyclases is so low (ca. 18-25% overall; Cunningham et al., 1994) that there is much uncertainty as to whether they share a common ancestry or, instead, represent an example of convergent evolution.

The need remains for the isolation of eukaryotic and prokaryotic genes and cDNAs encoding polypeptides involved in the carotenoid biosynthetic pathway, including those encoding a lycopene  $\epsilon$ -cyclase, IPP isomerase and  $\beta$ -carotene hydroxylase. There remains a need for methods to enhance the production of carotenoids, to alter the composition of carotenoids, and to reduce or eliminate carotenoid production. There also remains a need in the art for methods for screening for genes and cDNAs encoding enzymes of carotenoid biosynthesis and metabolism.

## SUMMARY OF THE INVENTION

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Accordingly, a first object of this invention is to provide purified and/or isolated nucleic acids which encode enzymes involved in carotenoid biosynthesis; in particular, lycopene  $\epsilon$ -cyclase, IPP isomerase and  $\beta$ -carotene hydroxylase.

A second object of this invention is to provide purified and/or isolated nucleic acids which encode enzymes which produce novel or uncommon carotenoids.

A third object of the present invention is to provide vectors containing said genes.

A fourth object of the present invention is to provide hosts transformed with said vectors.

Another object of the present invention is to provide hosts which accumulate novel or uncommon carotenoids or which accumulate greater amounts of specific or total carotenoids.

Another object of the present invention is to provide hosts with inhibited and/or altered carotenoid production.

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Another object of this invention is to secure the expression of eukaryotic carotenoid-related genes in a recombinant prokaryotic host.

Yet another object of the present invention is to provide a method for screening for eukaryotic and prokaryotic genes and cDNAs which encode enzymes involved in carotenoid biosynthesis and metabolism.

An additional object of the invention is to provide a method for manipulating carotenoid biosynthesis in photosynthetic organisms by inhibiting the synthesis of certain enzymatic products to cause accumulation of precursor compounds.

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Another object of the invention is to provide modified lycopene  $\epsilon$ -cyclase, IPP isomerase and  $\beta$ -carotene hydroxylase.

These and other objects of the present invention have been realized by the present inventors as described below.

A subject of the present invention is an isolated and/or purified nucleic acid sequence which encodes for a protein having lycopene  $\epsilon$ -cyclase, IPP isomerase or  $\beta$ -carotene hydroxylase enzyme activity and having the amino acid sequence of SEQ ID NOS: 2, 4, 14-21 or 23-27.

The invention also includes vectors which comprise any of the nucleic acid sequences listed above, and host cells transformed with such vectors.

Another subject of the present invention is a method of producing or enhancing the production of a carotenoid in a host cell, comprising inserting into the host cell a vector comprising a heterologous nucleic acid sequence which encodes for a protein having lycopene  $\epsilon$ -cyclase, IPP isomerase or  $\beta$ -carotene hydroxylase enzyme activity, wherein the heterologous nucleic acid sequence is operably linked to a promoter; and expressing the heterologous nucleic acid sequence to produce the protein.

Yet another subject of the present invention is a method of modifying the production of carotenoids in a host cell, the method comprising inserting into the host cell a vector comprising a heterologous nucleic acid sequence which produces an RNA and/or encodes for a protein which modifies lycopene  $\epsilon$ -cyclase, IPP isomerase or  $\beta$ -carotene hydroxylase enzyme activity, relative to an untransformed host cell, wherein the heterologous nucleic acid sequence is operably linked to a promoter; and expressing the heterologous nucleic acid sequence in the host cell to modify the production of the carotenoids in the host cell, relative to the untransformed host cell.

The present invention also includes a method of expressing, in a host cell, a heterologous nucleic acid sequence which encodes for a protein having lycopene  $\epsilon$ -cyclase, IPP isomerase or  $\beta$ -carotene hydroxylase enzyme activity, the method comprising inserting into the host cell a vector comprising the heterologous nucleic acid sequence, wherein the heterologous nucleic acid sequence is operably linked to a promoter; and expressing the heterologous nucleic acid sequence.

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Also included is a method of expressing, in a host cell, a heterologous nucleic acid sequence which encodes for a protein which modifies lycopene  $\epsilon$ -cyclase, IPP isomerase or  $\beta$ -carotene hydroxylase enzyme activity in the host cell, relative to an untransformed host cell, the method comprising inserting into the host cell a vector comprising the heterologous nucleic acid sequence, wherein the heterologous nucleic acid sequence is operably linked to a promoter; and expressing the heterologous nucleic acid sequence.

Another subject of the present invention is a method for screening for genes and cDNAs which encode enzymes involved in carotenoid biosynthesis and metabolism.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings, wherein:

Figure 1 is a schematic representation of the putative pathway of  $\beta$ -carotene biosynthesis in cyanobacteria, algae and plants. The enzymes catalyzing various steps are indicated at the left. Target sites of the bleaching herbicides NFZ and MPTA are also indicated at the left. Abbreviations: DMAPP, dimethylallyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; GPP, geranyl pyrophosphate; IPP, isopentenyl pyrophosphate; LCY, lycopene cyclase; MVA, mevalonic acid; MPTA, 2-(4-methylphenoxy)triethylamine hydrochloride; NFZ, norflurazon; PDS, phytoene desaturase; PSY, phytoene synthase; ZDS,  $\zeta$ -carotene desaturase; PPPP, prephytoene pyrophosphate.

Figure 2 depicts possible routes of synthesis of cyclic carotenoids and common plant and algal xanthophylls (oxycarotenolds) from neurosporene. Demonstrated activities of the  $\beta$ - and  $\epsilon$ -cyclase enzymes of A. thaliana are indicated by bold arrows labelled with  $\beta$  or  $\epsilon$  respectively. A bar below the arrow leading to  $\epsilon$ -carotene indicates that the enzymatic

activity was examined but no product was detected. The steps marked by an arrow with a dotted line have not been specifically examined. Conventional numbering of the carbon atoms is given for neurosporene and  $\alpha$ -carotene. Inverted triangles ( $\nabla$ ) mark positions of the double bonds introduced as a consequence of the desaturation reactions.

Figure 3 depicts the carotene endgroups which are found in plants.

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Figure 4 is a DNA sequence and the predicted amino acid sequence of a lycopene ε-cyclase cDNA isolated from A. thaliana (SEQ ID NOS: 1 and 2). These sequences were deposited under Genbank accession number U50738. This cDNA is incorporated into the plasmid pATeps.

Figure 5 is a DNA sequence encoding the  $\beta$ -carotene hydroxylase isolated from A. thaliana (SEQ ID NO: 3). This cDNA is incorporated into the plasmid pATOHB.

Figure 6 is an alignment of the predicted amino acid sequences of *A. thaliana* β-carotene hydroxylase (SEQ ID NO: 4) with those of the bacterial β-carotene hydroxylase enzymes from *Alicalgenes sp.* (SEQ ID NO: 5) (Genbank D58422), *Erwinia herbicola* Eho10 (SEQ ID NO.: 6) (GenBank M872280), *Erwinia uredovora* (SEQ ID NO.: 7) (GenBank D90087) and *Agrobacterium aurianticum* (SEQ ID NO.: 8) (GenBank D58420). A consensus sequence is also shown. All five genes are identical where a capital letter appears in the consensus. A lowercase letter indicates that three of five, including *A. thaliana*, have the identical residue. TM; transmembrane.

Figure 7 is a DNA sequence of a cDNA encoding an IPP isomerase isolated from A. thaliana (SEQ ID NO: 9). This cDNA is incorporated into the plasmid pATDP5.

Figure 8 is a DNA sequence of a second cDNA encoding another IPP isomerase isolated from A. thaliana (SEQ ID NO: 10). This cDNA is incorporated into the plasmid pATDP7.

Figure 9 is a DNA sequence of a cDNA encoding an IPP isomerase isolated from *Haematococcus pluvialis* (SEQ ID NO: 11). This cDNA is incorporated into the plasmid pHP04.

Figure 10 is a DNA sequence of a second cDNA encoding another IPP isomerase isolated from *Haematococcus pluvialis* (SEQ ID NO: 12). This cDNA is incorporated into the plasmid pHP05.

Figure 11 is an alignment of the amino acid sequences predicted by IPP isomerase cDNAs isolated from A. thaliana (SEQ ID NO.: 16 and 18), H. pluvialis (SEQ ID NOS.: 14

and 15), Clarkia breweri (SEQ ID NO.: 17) (See, Blanc & Pichersky, Plant Physiol. (1995) 108:855; Genbank accession no. X82627) and Saccharomyces cerevisiae (SEQ ID NO.: 19) (Genbank accession no. J05090).

Figure 12 is a DNA sequence of the cDNA encoding an IPP isomerase isolated from *Tagetes erecta* (marigold; SEQ ID NO: 13). This cDNA is incorporated into the plasmid pPMDP1. xxx's denote a region not originally sequenced. Figure 21A shows the complete marigold sequence.

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Figure 13 is an alignment of the consensus sequence of four plant  $\beta$ -cyclases (SEQ ID NO.: 20) with the A. thaliana lycopene  $\epsilon$ -cyclase (SEQ ID NO.: 21). A capital letter in the plant  $\beta$  consensus is used where all four  $\beta$ -cyclase genes predict the same amino acid residue in this position. A small letter indicates that an identical residue was found in three of the four. Dashes indicate that the amino acid residue was not conserved and dots in the sequence denote a gap. A consensus for the aligned sequences is given, in capital letters below the alignment, where the  $\beta$ - and  $\epsilon$ -cyclases have the same amino acid residue. Arrows indicate some of the conserved amino acids that will be used as junction sites for construction of chimeric cyclases with novel enzymatic activities. Several regions of interest including a sequence signature indicative of a dinucleotide-binding motif and two predicted transmembrane (TM) helical regions are indicated below the alignment and are underlined.

Figure 14 shows the nucleotide (SEQ ID NO:22) and amino acid sequences (SEQ ID NO:23) of the *Adonis palaestina* (pheasant's eye)  $\epsilon$ -cyclase cDNA #5.

Figure 15A shows the nucleotide (SEQ ID NO:24) and amino acid sequences (SEQ ID NO:25) of a potato ε-cyclase cDNA. Figure 15B shows the amino acid sequence (SEQ ID NO:26) of a chimeric lettuce/potato lycopene ε-cyclase. Amino acids in lower case are from the lettuce cDNA and those in upper case are from the potato cDNA. The product of this chimeric cDNA has e-cyclase activity and converts lycopene to the monocyclic δ-carotene.

Figure 16 shows a comparison between the amino acid sequences of the *Arabidopsis* ∈-cyclase (SEQ ID NO:27) and the potato ∈-cyclase (SEQ ID NO:25).

Figure 17A shows the nucleotide sequence of the *Adonis palaestina* Ipi1 (SEQ ID NO:28) and Figure 17B shows the nucleotide sequence of the *Adonis palaestina* Ipi2 (SEQ ID NO: 29).

Figure 18A shows the nucleotide sequence of the *Haematoccus pluvialis* Ipi1 (SEQ ID NO:11) and Figure 18B shows the nucleotide sequence of the *Haematoccus pluvialis* Ipi2 (SEQ ID NO:30).

Figure 19A shows the nucleotide sequence of the *Lactuca sativa (romaine lettuce)*Ipi1 (SEQ ID NO:31) and Figure 19B shows the nucleotide sequence of the *Lactuca sativa*Ipi2 (SEQ ID NO: 32).

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Figure 20 shows the nucleotide sequence of the *Chlamydomonas reinhardtii* Ipi1 (SEO ID NO:33).

Figure 21A shows the nucleotide sequence of the *Tagetes erecta* (marigold) Ipi1 (SEQ ID NO:34) and Figure 21B shows the nucleotide sequence of the *Oryza sativa* (rice) Ipi1 (SEQ ID NO:35).

Figure 22 shows a amino acid sequence alignment of various plant and green algal isopentenyl isomerases (IPI) (SEQ ID NOS:16, 36-45).

Figure 23 shows a comparison between *Adonis palaestina* ∈-cyclase cDNA #3 and cDNA #5 nucleotide sequences.

Figure 24 shows a comparison between *Adonis palaestina* ∈-cyclase cDNA #3 and cDNA #5 predicted amino acid sequences.

Figure 25 shows a sequence alignment of various plant  $\beta$ - and  $\epsilon$ -cyclases. Those sequences outlined in grey denote identical sequences among the  $\epsilon$ -cyclases. Those sequences outlined in black denote identical sequences among both the  $\beta$ - and  $\epsilon$ -cyclases.

Figure 26 shows a sequence alignment of the plant  $\epsilon$ -cyclases from Figure 25. Those sequences outlined in black denote identical sequences among the  $\epsilon$ -cyclases.

Figure 27 is a dendrogram or "tree" illustrating the degree of amino acid sequence similarity for various lycopene  $\beta$ - and  $\epsilon$ -cyclases.

Figure 28 shows a comparison between Arabidopsis  $\epsilon$ -cyclase and lettuce  $\epsilon$ -cyclase predicted amino acid sequences.

# DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention includes an isolated and/or purified nucleic acid sequence which encodes for a protein having lycopene  $\epsilon$ -cyclase, IPP isomerase or  $\beta$ -carotene hydroxylase enzyme activity and having the amino acid sequence of SEQ ID NOS: 2, 4, 14-21, 23 or 25-27. Nucleic acids encoding lycopene  $\epsilon$ -cyclase,  $\beta$ -carotene hydroxylase and IPP

isomerases have been isolated from several genetically distant sources.

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The present inventors have isolated nucleic acids encoding the enzyme IPP isomerase, which catalyzes the reversible conversion of isopentenyl pyrophosphate (IPP) to dimethylallyl pyrophosphate (DMAPP). IPP isomerase cDNAs were isolated from the plants A. thaliana, Tagetes erecta (marigold), Adonis palaestina (pheasant's eye), Lactuca sativa (romaine lettuce) and from the green algae H. pluvialis and Chlamydomonas reinhardtii. Alignments of the amino acid sequences predicted by some of these cDNAs are shown in Figures 12 and 22. Plasmids containing some of these cDNAs were deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville MD 20852 on March 4, 1996 under ATCC accession numbers 98000 (pHP05 - H. pluvialis); 98001 (pMDP1 - marigold); 98002 (pATDP7 - A. thaliana) and 98004 (pHP04 - H. pluvialis).

The present inventors have also isolated nucleic acids encoding the enzyme  $\beta$ -carotene hydroxylase, which is responsible for hydroxylating the  $\beta$ -endgroup in carotenoids. The nucleic acid of the present invention is shown in SEQ ID NO: 3 and Figure 5. The full length cDNA product hydroxylates both end groups of  $\beta$ -carotene as do products of cDNAs which encode proteins truncated by up to 50 amino acids from the N-terminus. Products of genes which encode proteins truncated between about 60-110 amino acids from the N-terminus preferentially hydroxylate only one ring. A plasmid containing this gene was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville MD 20852 on March 4, 1996 under ATCC accession number 98003 (pATOHB - A. thaliana).

The present inventors have also isolated nucleic acids encoding the enzyme lycopene  $\epsilon$ -cyclase, which is responsible for the formation of  $\epsilon$ -endgroups in carotenoids. The A. thaliane  $\epsilon$ -cyclase adds an  $\epsilon$  ring to only one end of the symmetrical lycopene while the related  $\beta$ -cyclase adds a ring at both ends. The A. thaliana cDNA of the present invention is shown in Figure 4 and SEQ ID NO: 1. A plasmid containing this gene was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville MD 20852 on March 4, 1996 under ATCC accession number 98005 (pATeps - A. thaliana).

In addition, lycopene  $\epsilon$ -cyclases have been identified in lettuce and in *Adonis* palaestina (cDNA #5) which encode enzymes that convert lycopene to the bicyclic  $\epsilon$ -carotene ( $\epsilon$ , $\epsilon$ -carotene). An additional cDNA from *Adonis palaestina* (cDNA #3) encodes a lycopene  $\epsilon$ -cyclase which converts lycopene into  $\delta$ -carotene ( $\epsilon$ , $\psi$ -carotene) and differs from the lycopene  $\epsilon$ -cyclase which forms bicyclic  $\epsilon$ -carotene ( $\epsilon$ , $\epsilon$ -carotene) by only 5 amino acids.

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One or more of these amino acids may be modified by alteration of the nucleotide sequence in the #5 cDNA to obtain an enzyme which forms the bicyclic  $\epsilon, \epsilon$ -carotene. The sequences of the Adonis palaestina and Arabidopsis thaliana ∈-cyclases have about 70% nucleotide identity and about 72% amino acid identity.

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Initial experiments by the inventors with chimeric genes indicated that the part of the  $\epsilon$ -cyclase which is responsible for adding  $2 \epsilon$  rings to form  $\epsilon, \epsilon$ -carotene is the carboxy terminal portion of the gene. The lettuce  $\epsilon$ -cyclase adds two  $\epsilon$  rings to form  $\epsilon$ ,  $\epsilon$ -carotene. A DNA encoding a partial potato  $\epsilon$ -cyclase (missing its amino terminal portion), when combined with an amino terminal region from the lettuce  $\epsilon$ -cyclase gene, produces a monocyclic  $\delta$ -carotene ( $\epsilon$ , $\psi$ -carotene). With the discovery of the differences between the Adonis palaestina clone #3 and clone #5, the specific amino acids responsible for the addition of an extra  $\epsilon$  ring have been identified (Figure 24). Specifically, amino acid 55 is Thr in clone #3 and Ser in clone #5, amino acid 210 is Asn in clone #3 and Asp in clone #5, amino acid 231 is Asp in clone #3 and Glu in clone #5, amino acid 352 is Ile in clone #3 and Val in clone #5, and amino acid 524 is Lys in clone #3 and Arg in clone #5. It can be appreciated that these changes are quite conservative, as only one change, at amino acid 210, changes the charge of the protein.

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presently disclosed may be altered to increase a particularly desirable property of the enzyme, to change a property of the enzyme, or to diminish an undesirable property of the enzyme. Such modifications can be by deletion, substitution, or insertion of one or more amino acids, and can be performed by routine enzymatic manipulation of the nucleic acid encoding the enzyme (such as by restriction enzyme digestion, removal of nucleotides by mung bean nuclease or Bal31, insertion of nucleotides by Klenow fragment, and by religation of the ends), by site-directed mutagenesis, or may be accidental, such as by low fidelity PCR or those obtained through mutations in hosts that are producers of the enzymes. These techniques as well as other suitable techniques are well known in the art.

Thus, it is clear that the nucleic acids of the invention encoding the enzymes as

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Mutations can be made in the nucleic acids of the invention such that a particular codon is changed to a codon which codes for a different amino acid. Such a mutation is generally made by making the fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a nonconservative manner (i.e., by changing the codon from an amino acid belonging to a grouping 5

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of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting protein.

The following is one example of various groupings of amino acids:

Amino acids with nonpolar R groups: Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Tryptophan and Methionine.

Amino acids with uncharged polar R groups: Glycine, Serine, Threonine, Cysteine, Tyrosine, Asparagine and Glutamine.

Amino acids with charged polar R groups (negatively charged at Ph 6.0): Aspartic acid and Glutamic acid.

Basic amino acids (positively charged at pH 6.0): Lysine, Arginine and Histidine.

Another grouping may be those amino acids with phenyl groups: Phenylalanine, Tryptophan and Tyrosine.

Another grouping may be according to molecular weight (i.e., size of R groups). Particularly preferred substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;

- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- Gln for Asn such that a free NH<sub>2</sub> can be maintained.

Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced to provide a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (i.e., His can act as an acid or base and is the most common amino acid in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces  $\beta$ -turns in the protein's structure.

It is clear that certain modifications of SEQ ID NOS: 2, 4, 14-21, 23 or 25-27 can take place without destroying the activity of the enzyme. It is noted especially that truncated

versions of the nucleic acids of the invention are functional. For example, several amino acids (from 1 to about 120) can be deleted from the N-terminus of the lycopene  $\epsilon$ -cyclases of the invention, and a functional protein can still be produced. This fact is made especially clear from Figure 25, which shows a sequence alignment of several plant  $\epsilon$ -cyclases. As can be seen from Figure 25, there is an enormous amount of sequence disparity between amino acid sequences 2 to about 50-70 (depending on the particular sequence, since gaps are present). There is less, but also a substantial amount of, sequence dissimilarity between about 50-70 to about 90-120 (depending on the particular sequence). Thereafter, the sequences are fairly conserved, except for small pockets of dissimilarity between about 275-295 to about 285-305 (depending on the particular sequence), and between about 395-415 to about 410-430 (depending on the particular sequence).

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The present inventors have found that the amount of the 5' region present in the nucleic acids of the invention can alter the activity of the enzyme. Instead of diminishing activity, truncating the 5' region of the nucleic acids of the invention may result in an enzyme with a different specificity. Thus, the present invention relates to nucleic acids and enzymes encoded thereby which are truncated to within 0-50, preferably 0-25, codons of the 5' initiation codon of their prokaryotic counterparts as determined by alignment maps as discussed below.

For example, when the cDNA encoding A. thaliana  $\beta$ -carotene hydroxylase was truncated, the resulting enzyme catalyzed the formation of  $\beta$ -cryptoxanthin as the major product and zeaxanthin as minor product; in contrast to its normal production of zeaxanthin.

The present invention is intended to include those nucleic acid and amino acid sequences in which substitutions, deletions, additions or other modifications have taken place, as compared to SEQ ID NOS: 2, 4, 14-21, 23 or 25-27, without destroying the activity of the enzyme. Preferably, the substitutions, deletions, additions or other modifications take place at the 5' end, or any other of those positions which already show dissimilarity between any of the presently disclosed amino acid sequences (see also Figure 25) or other amino acid sequences which are known in the art and which encode the same enzyme (i.e., lycopene  $\epsilon$ -cyclase, IPP isomerase or  $\beta$ -carotene hydroxylase).

In each case, nucleic acid and amino acid sequence similarity and identity is measured using sequence analysis software, for example, the Sequence Analysis, Gap, or BestFit software packages of the Genetics Computer Group (University of Wisconsin Biotechnology

Center, 1710 University Avenue, Madison, Wisconsin 53705), MEGAlign (DNAStar, Inc., 1228 S. Park St., Madison, Wisconsin 53715), or MacVector (Oxford Molecular Group, 2105 S. Bascom Avenue, Suite 200, Campbell, California 95008). Such software uses algorithms to match similar sequences by assigning degrees of identity to various substitutions, deletions, and other modifications, and includes detailed instructions as to useful parameters, etc., such that those of routine skill in the art can easily compare sequence similarities and identities. An example of a useful algorithm in this regard is the algorithm of Needleman and Wunsch, which is used in the Gap program discussed above. This program finds the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. Another useful algorithm is the algorithm of Smith and Waterman, which is used in the BestFit program discussed above. This program creates an optimal alignment of the best segment of similarity between two sequences. Optimal alignments are found by inserting gaps to maximize the number of matches using the local homology algorithm of Smith and Waterman.

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Conservative (i.e. similar) substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine and leucine; aspartic acid, glutamic acid, asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Substitutions may also be made on the basis of conserved hydrophobicity or hydrophilicity (see Kyte and Doolittle, *J. Mol. Biol.* 157: 105-132 (1982)), or on the basis of the ability to assume similar polypeptide secondary structure (see Chou and Fasman, *Adv. Enzymol.* 47: 45-148 (1978)).

If comparison is made between nucleotide sequences, preferably the length of comparison sequences is at least 50 nucleotides, more preferably at least 60 nucleotides, at least 75 nucleotides or at least 100 nucleotides. It is most preferred if comparison is made between the nucleic acid sequences encoding the enzyme coding regions necessary for enzyme activity. If comparison is made between amino acid sequences, preferably the length of comparison is at least 20 amino acids, more preferably at least 30 amino acids, at least 40 amino acids or at least 50 amino acids. It is most preferred if comparison is made between the amino acid sequences in the enzyme coding regions necessary for enzyme activity.

It should be appreciated that also within the scope of the present invention are nucleic acid sequences encoding lycopene  $\epsilon$ -cyclases, IPP isomerases and  $\beta$ -carotene hydroxylases

which code for enzymes having the same amino acid sequence as SEQ ID NOS: 2, 4, 14-21, 23 or 25-27, but which are degenerate to the nucleic acids specifically disclosed herein.

The amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property of immunoglobulin-binding is retained by the polypeptide.

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In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994))]; "Current Protocols in Immunology" Volumes I-III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

The present invention also includes vectors. Suitable vectors according to the present invention comprise a nucleic acid of the invention encoding an enzyme involved in carotenoid biosynthesis or metabolism and a suitable promoter for the host, and can be constructed using techniques well known in the art (for example Sambrook et al., Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York, 1991). Suitable vectors for eukaryotic expression in plants are described in Frey et al., Plant J. (1995) 8(5):693 and Misawa et al, 1994a; incorporated herein by reference. Suitable vectors for prokaryotic expression include pACYC184, pUC119, and pBR322 (available from New England BioLabs, Bevery, MA) and pTrcHis (Invitrogen) and pET28 (Novagen) and derivatives thereof. The vectors of the present invention can additionally contain regulatory elements such as promoters, repressors, selectable markers such as antibiotic resistance genes, etc.

The nucleic acids encoding the carotenoid enzymes as described above, when cloned into a suitable expression vector, can be used to overexpress these enzymes in a plant

expression system or to inhibit the expression of these enzymes. For example, a vector containing the gene encoding lycopene  $\epsilon$ -cyclase can be used to increase the amount of  $\alpha$ -carotene and carotenoids derived from  $\alpha$ -carotene (such as lutein and  $\alpha$ -cryptoxanthin) in an organism and thereby alter the nutritional value, pharmacology and visual appearance value of the organism.

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Therefore, the present invention includes a method of producing or enhancing the production of a carotenoid in a host cell, relative to an untransformed host cell, the method comprising inserting into the host cell a vector comprising a heterologous nucleic acid sequence which encodes for a protein having lycopene  $\epsilon$ -cyclase, IPP isomerase or  $\beta$ -carotene hydroxylase enzyme activity, wherein the heterologous nucleic acid sequence is operably linked to a promoter; and expressing the heterologous nucleic acid sequence to produce the protein.

The present invention also includes a method of modifying the production of carotenoids in a host cell, the method comprising inserting into the host cell a vector comprising a heterologous nucleic acid sequence which produces an RNA and/or encodes for a protein which modifies lycopene ε-cyclase, IPP isomerase or β-carotene hydroxylase enzyme activity, relative to an untransformed host cell, wherein the heterologous nucleic acid sequence is operably linked to a promoter; and expressing the heterologous nucleic acid sequence in the host cell to modify the production of the carotenoids in the host cell, relative to the untransformed host cell.

The term "modifying the production" means that the amount of carotenoids produced in the host cell can be enhanced, reduced, or left the same, as compared to the untransformed host cell. In accordance with one embodiment of the present invention, the make-up of the carotenoids (i.e., the specific carotenoids produced) is changed vis a vis each other, and this change in make-up may result in either a net gain, net loss, or no net change in the total amount of carotenoids produced in the cell. In accordance with another embodiment of the present invention, the production or the biochemical activity of the carotenoids (or the enzymes which catalyze their formation) is enhanced by the insertion of an enzyme-encoding nucleic acid of the invention. In yet another embodiment of the invention, the production or the biochemical activity of the carotenoids (or the enzymes which catalyze their formation) may be reduced or inhibited by a number of different approaches available to those skilled in the art, including but not limited to such methodologies or approaches as anti-sense (e.g.,

Gray et al (1992) Plant Mol. Biol. 19:69-87), ribozymes (e.g., Wegener et al (1994) Mol. Gen. Genet. 245:465-470), co-suppression (e.g., Fray and Grierson (1993) Plant Mol. Biol. 22:589-602), targeted disruption of the gene (e.g., Schaefer et al. (1997) Plant J. 11:1195-1206), intracellular antibodies (e.g., Rondon and Marasco (1997) Ann. Rev. Microbiol. 51:257-283) or whatever other approaches rely on the knowledge or availability of the nucleic acid or amino acid sequences of the invention and/or portions thereof, to thereby reduce accumulation of carotenoids with  $\epsilon$  rings and compounds derived from them (for  $\epsilon$ -cyclase inhibition), or carotenoids with hydroxylated  $\beta$  rings and compounds derived from them (for  $\beta$ -hydroxylase inhibition), or, in the case if IPP isomerase, accumulation of any isoprenoid compound.

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Preferably, at least a portion of the nucleic acid sequences used in the methods, vectors and host cells of the invention codes for an enzyme having an amino acid sequence which is at least 85% identical, preferably at least 90%, at least 95% or completely identical to SEQ ID NOS: 2, 4, 14-21, 23 or 25-27. Sequence identity is determined as noted above. Preferably, sequence additions, deletions or other modifications are made as indicated above, so as to not affect the function of the particular enzyme.

In a preferred embodiment, vectors are manufactured which contain a DNA encoding a eukaryotic IPP isomerase upstream of a DNA encoding a second eukaryotic carotenoid enzyme. The inventors have discovered that inclusion of an IPP isomerase gene increases the supply of substrate for the carotenoid pathway; thereby enhancing the production of carotenoid endproducts, as compared to a host cell which is not transformed with such a vector. This is apparent from the much deeper pigmentation in carotenoid-accumulating colonies of E. coli which also contain one of the aforementioned IPP isomerase genes when compared to colonies that lack this additional IPP isomerase gene. Similarly, a vector comprising an IPP isomerase gene can be used to enhance production of any secondary metabolite of dimethylallyl pyrophosphate and/or isopentenyl pyrophosphate (such as isoprenoids, steroids, carotenoids, etc.). The term "isoprenoid" is intended to mean any member of the class of naturally occurring compounds whose carbon skeletons are composed, in part or entirely, of isopentyl  $C_5$  units. Preferably, the carbon skeleton is of an essential oil, a fragrance, a rubber, a carotenoid, or a therapeutic compound, such as paclitaxel.

A vector containing the cDNA encoding a lycopene  $\epsilon$ -cyclase of the invention, preferably the lettuce lycopene  $\epsilon$ -cyclase or Adonis  $\epsilon$ -cyclase #5, can be used to increase the

amount of bicyclic ∈-carotene in an organism and thereby alter the nutritional value, pharmacology and visual appearance value of the organism. In addition, the transformed organism can be used in the formulation of therapeutic agents, for example in the treatment of cancer (see Mayne et al (1996) FASEB J. 10:690-701; Tsushima et al (1995) Biol. Pharm. Bull. 18:227-233).

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An antisense strand of a nucleic acid of the invention can be inserted into a vector. For example, the lycopene  $\epsilon$ -cyclase gene can be inserted into a vector and incorporated into the genomic DNA of a host, thereby inhibiting the synthesis of  $\epsilon$ , $\beta$ -carotenoids (lutein and  $\alpha$ -carotene) and enhancing the synthesis of  $\beta$ , $\beta$ -carotenoids (zeaxanthin and  $\beta$ -carotene).

The present invention also relates to novel enzymes which are encoded by the amino acid sequences of the invention, or portions thereof.

The present invention also relates to novel enzymes which can transform known carotenoids into novel or uncommon products. Currently  $\epsilon$ -carotene (see Figure 2) and  $\gamma$ -carotene are commonly produced only in minor amounts. As described below, an enzyme can be produced which transforms lycopene to  $\gamma$ -carotene and lycopene to  $\epsilon$ -carotene. With these products in hand, bulk synthesis of other carotenoids derived from them are possible. For example,  $\epsilon$ -carotene can be hydroxylated to form lactucaxanthin, an isomer of lutein (one  $\epsilon$  and one  $\beta$  ring) and zeaxanthin (two  $\beta$  rings) where both endgroups are, instead,  $\epsilon$  rings.

In addition to novel enzymes produced by truncating the 5' region of known enzymes, as discussed above, novel enzymes which can participate in the formation of unusual carotenoids can be formed by replacing portions of one gene with an analogous sequence from a structurally related gene. For example,  $\beta$ -cyclase and  $\epsilon$ -cyclase are structurally related (see Figure 13). By replacing a portion of  $\beta$ -lycopene cyclase with the analogous portion of  $\epsilon$ -cyclase, an enzyme which produces  $\gamma$ -carotene will be produced (one  $\beta$  endgroup). Further, by replacing a portion of the lycopene  $\epsilon$ -cyclase with the analogous portion of  $\beta$ -cyclase, an enzyme which produces  $\epsilon$ -carotene will be produced (with some exceptions, such as the lettuce  $\epsilon$ -cyclase, plant  $\epsilon$ -cyclases normally produce a compound with one  $\epsilon$ -endgroup,  $\delta$ -carotene). Similarly,  $\beta$ -hydroxylase could be modified to produce enzymes of novel function by creation of hybrids with  $\epsilon$ -hydroxylase.

Host systems according to the present invention can comprise any organism that already produces carotenoids or which has been genetically modified to produce carotenoids.

The IPP isomerase genes are more broadly applicable for enhancing production of any product dependent on DMAPP and/or IPP as a precursor.

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Organisms which already produce carotenoids include plants, algae, some yeasts, fungi and cyanobacteria and other photosynthetic bacteria. Transformation of these hosts with vectors according to the present invention can be done using standard techniques such as those described in Misawa et al., (1990) supra; Hundle et al., (1993) supra; Hundle et al., (1991) supra; Misawa et al., (1991) supra; Sandmann et al., supra; and Schnurr et al., supra.

Transgenic organisms can be constructed which include the nucleic acid sequences of the present invention (Bird et al, 1991; Bramley et al, 1992; Misawa et al, 1994a; Misawa et al, 1994b; Cunningham et al, 1993). The incorporation of these sequences can allow the controlling of carotenoid biosynthesis, content, or composition in the host cell. These transgenic systems can be constructed to incorporate sequences which allow for the overexpression of the nucleic acids of the present invention. Transgenic systems can also be constructed containing antisense expression of the nucleic acid sequences of the present invention. Such antisense expression would result in the accumulation of the substrates of the substrates of the enzyme encoded by the sense strand.

A method for screening for eukaryotic genes which encode enzymes involved in carotenoid biosynthesis comprises transforming a prokaryotic host with a nucleic acid which may contain a eukaryotic or prokaryotic carotenoid biosynthetic gene; culturing said transformed host to obtain colonies; and screening for colonies exhibiting a different color than colonies of the untransformed host.

Suitable hosts include *E. coli*, cyanobacteria such as *Synechococcus* and *Synechocystis*, alga and plant cells. *E. coli* are preferred.

In a preferred embodiment, the above "color complementation" screening protocol can be enhanced by using mutants which are either (1) deficient in at least one carotenoid biosynthetic gene or (2) overexpress at least one carotenoid biosynthetic gene. In either case, such mutants will accumulate carotenoid precursors.

Prokaryotic and eukaryotic DNA or cDNA libraries can be screened in total for the presence of genes of carotenoid biosynthesis, metabolism and degradation. Preferred organisms to be screened include photosynthetic organisms.

E. coli can be transformed with these eukaryotic cDNA libraries using conventional methods such as those described in Sambrook et al, 1989 and according to protocols described by the vendors of the cloning vectors.

For example, the cDNA libraries in bacteriophage vectors such as lambdaZAP (Stratagene) or lambda ZIPLOX (Gibco BRL) can be excised en masse and used to transform *E.coli*.

Transformed *E. coli* can be cultured using conventional techniques. The culture broth preferably contains antibiotics to select and maintain plasmids. Suitable antibiotics include penicillin, ampicillin, chloramphenicol, etc. Culturing is typically conducted at 15-40°C, preferably at room temperature or slightly above (18-28°C), for 12 hours to 7 days.

Cultures are plated and the plates are screened visually for colonies with a different color than the colonies of the host  $E.\ coli$  transformed with the empty plasmid cloning vector. For example,  $E.\ coli$  transformed with the plasmid, pAC-BETA (described below), produce yellow colonies that accumulate  $\beta$ -carotene. After transformation with a cDNA library, colonies which contain a different hue than those formed by  $E.\ coli/pAC$ -BETA would be expected to contain enzymes which modify the structure or accumulation of  $\beta$ -carotene. Similar  $E.\ coli$  strains can be engineered which accumulate earlier products in carotenoid biosynthesis, such as lycopene,  $\gamma$ -carotene, etc.

Having generally described this invention, a further understanding can be obtained by reference to certain specific examples which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

#### **EXAMPLE**

#### I. Isolation of β-carotene hydroxylase

#### **Plasmid Construction**

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An 8.6kb BgIII fragment containing the carotenoid biosynthetic genes of *Erwinia herbicola* was first cloned in the BamHI site of plasmid vector pACYC184 (chloramphenicol resistant), and then a 1.1kb BamHI fragment containing the *E. herbicola* β-carotene hydroxylase (*CrtZ*) was deleted. *E.coli* strains containing the resulting plasmid, pAC-BETA, accumulate β-carotene and form yellow colonies (Cunningham et al., 1994).

A full length cDNA encoding IPP isomerase of *Haematococcus pluvialis* (HP04) was first excised with *BamH*I and *Kpn*I from pBluescript SK-, and then ligated into the

corresponding sites of the pTrcHisA vector with high-level expression from the *trc* promoter (Invitrogen, Inc.). A fragment containing the IPP isomerase and *trc* promoter was subsequently excised with *EcoRV* and *KpnI*, treated with the Klenow fragment of DNA polymerase to produce blunt ends, and ligated in the Klenow-treated *HindIII* site of pAC-BETA. *E.coli* cells transformed with this new plasmid pAC-BETA-04 form orange colonies on LB plates (*vs.* yellow for those containing pAC-BETA) and cultures accumulate substantially more β-carotene (*ca.* two fold) than those that contain pAC-BETA.

#### Screening of an Arabidopsis cDNA Library

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Several  $\lambda$  cDNA expression libraries of *Arabidopsis* were obtained from the *Arabidopsis* Biological Resource Center (Ohio State University, Columbus, OH) (Kieber et al., 1993). The  $\lambda$  cDNA libraries were excised *in vivo* using Stratagene's ExAssist SOLR system to produce a phagemid cDNA library wherein each phagemid contained also a gene conferring resistance to the antibiotic ampicillin.

E.coli strain DH10BZIP was chosen as the host cell for the screening and pigment production, although we have also used TOP10F' and XL1-Blue for this purpose. DH10B cells were transformed with plasmid pAC-BETA-04 and were plated on LB agar plates containing chloramphenicol at 50 µg/ml (from United States Biochemical Corporation). The phagemid Arabidopsis cDNA library was then introduced into DH10B cells already containing pAC-BETA-04. Transformed cells containing both pAC-BETA-04 and Arabidopsis cDNA library phagemids were selected on chloramphenical plus ampicillin (150 µg/ml) agar plates. Maximum color development occurred after 3 to 7 days incubation at room temperature, and the rare bright yellow colonies were selected from a background of many thousands of orange colonies on each agar plate. Selected colonies were inoculated into 3 ml liquid LB medium containing ampicillin and chloramphenicol, and cultures were incubated at room temperature for 1-2 days, with shaking. Cells were then harvested by centrifugation and extracted with acetone in microfuge tubes. After centrifugation, the pigmented extract was spotted onto silica gel thin-layer chromatography (TLC) plates, and developed with a hexane:ether (1:1, by volume) mobile phases. B-carotene hydroxylaseencoding cDNAs were identified based on the appearance of a yellow pigment that comigrated with zeaxanthin on the TLC plates.

#### Subcloning and Sequencing

The plasmid containing the β-carotene hydroxylase cDNA was recovered and analyzed by standard procedures (Sambrook et al., 1989). The *Arabidopsis* β-carotene hydroxylase was sequenced completely on both strands on an automatic sequencer (Applied Biosystems, Model 373A, Version 2.0.1S). The cDNA insert of 0.95kb also was excised and ligated into the a pTrcHis vector. A *BgI*II restriction site within the cDNA was used to remove that portion of the cDNA that encodes the predicted polypeptide N terminal sequence region that is not also found in bacterial β-carotene hydroxylases (Figure 6). A BgIII-XhoI fragment was directionally cloned in BamHI-XhoI digested TrcHis vectors.

#### 10 Pigment Analysis

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A single colony was used to inoculate 50 ml of LB containing ampicillin and chloramphenicol in a 250-ml flask. Cultures were incubated at 28°C for 36 hours with gentle shaking, and then harvested at 5000 rpm in an SS-34 rotor. The cells were washed once with distilled H<sub>2</sub>O and resuspended with 0.5 ml of water. The extraction procedures and HPLC were essentially as described previously (Cunningham et al, 1994).

# II. Isolation and biochemical analysis of an Arabidopsis lycopene $\epsilon$ -cyclase Plasmid Construction

Construction of plasmids pAC-LYC, pAC-NEUR, and pAC-ZETA is described in Cunningham et al., (1994). In brief, the appropriate carotenoid biosynthetic genes from *Erwinia herbicola*, *Rhodobacter capsulatus*, and *Synechococcus* sp. strain PCC7942 were cloned in the plasmid vector pACYC184 (New England BioLabs, Beverly, MA). Cultures of *E. coli* containing the plasmids pAC-ZETA, pAC-NEUR, and pAC-LYC, accumulate ζ-carotene, neurosporene, and lycopene, respectively. The plasmid pAC-ZETA was constructed as follows: an 8.6-kb BgIII fragment containing the carotenoid biosynthetic genes of *E. herbicola* (GenBank M87280; Hundle et al., 1991) was obtained after partial digestion of plasmid pPL376 (Perry et al., 1986; Tuveson et al., 1986) and cloned in the BamHI site of pACYC184 to give the plasmid pAC-EHER. Deletion of adjacent 0.8- and 1.1-kb BamHI-BamHI fragments (deletion Z in Cunningham et al., 1994), and of a 1.1 kB SalI-SalI fragment (deletion X) served to remove most of the coding regions for the *E. herbicola* β-carotene hydroxylase (crtZ gene) and zeaxanthin glucosyltransferase (crtX gene), respectively. The

resulting plasmid, pAC-BETA, retains functional genes for geranylgeranyl pyrophosphate synthase (crtE), phytoene synthase (crtB), phytoene desaturase (crtI), and lycopene cyclase (crtY). Cells of *E. coli* containing this plasmid form yellow colonies and accumulate  $\beta$ -carotene. A plasmid containing both the lycopene  $\epsilon$ - and  $\beta$ -cyclase cDNAs of *A. thaliana* was constructed by excising the  $\epsilon$ -cyclase in clone y2 as a PvuI-PvuII fragment and ligating this piece in the SnaBI site of a plasmid (pSPORT 1 from GIBCO-BRL) that already contained the  $\beta$ -cyclase (Cunningham et al., 1996).

#### **Organisms and Growth Conditions**

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E. coli strains TOP10 and TOP10 F' (obtained from Invitrogen Corporation, San Diego, CA) and XL1-Blue (Stratagene) were grown in Luria-Bertani (LB) medium (Sambrook et al., 1989) at 37°C in darkness on a platform shaker at 225 cycles per min. Media components were from Difco (yeast extract and tryptone) or Sigma (NaCl). Ampicillin at 150 μg/mL and/or chloramphenicol at 50 μg/mL (both from United States Biochemical Corporation) were used, as appropriate, for selection and maintenance of plasmids.

#### Mass Excision and Color Complementation Screening of an A. thaliana cDNA Library

A size-fractionated 1-2 kB cDNA library of A. thaliana in lambda ZAPII (Kieber et al., 1993) was obtained from the Arabidopsis Biological Resource Center at The Ohio State University (stock number CD4-14). Other size fractionated libraries were also obtained (stock numbers CD4-13, CD4-15, and CD4-16). An aliquot of each library was treated to cause a mass excision of the cDNAs and thereby produce a phagemid library according to the instructions provided by the supplier of the cloning vector (Stratagene; E. coli strain XL1-Blue and the helper phage R408 were used). The titre of the excised phagemid was determined and the library was introduced into a lycopene-accumulating strain of E. coli TOP10 F' (this strain contained the plasmid pAC-LYC) by incubation of the phagemid with the E. coli cells for 15 min at 37°C. Cells had been grown overnight at 30°C in LB medium supplemented with 2% (w/v) maltose and 10 mM MgSO<sub>4</sub> (final concentration), and harvested in 1.5 ml microfuge tubes at a setting of 3 on an Eppendorf microfuge (5415C) for 10 min. The pellets were resuspended in 10 mM MgSO<sub>4</sub> to a volume equal to one-half that of the

initial culture volume. Transformants were spread on large (150 mm diameter) LB agar petri plates containing antibiotics to provide for selection of cDNA clones (ampicillin) and maintenance of pAC-LYC (chloramphenicol). Approximately 10,000 colony forming units were spread on each plate. Petri plates were incubated at 37.C for 16 hr and then at room temperature for 2 to 7 days to allow maximum color development. Plates were screened visually with the aid of an illuminated 3x magnifier and a low power stage-dissecting microscope for the rare, pale pinkish-yellow to deep-yellow colonies that could be observed in the background of pink colonies. A colony color of yellow or pinkish-yellow was taken as presumptive evidence of a cyclization activity. These yellow colonies were collected with sterile toothpicks and used to inoculate 3ml of LB medium in culture tubes with overnight growth at 37°C and shaking at 225 cycles/min. Cultures were split into two aliquots in microfuge tubes and harvested by centrifugation at a setting of 5 in an Eppendorf 5415C microfuge. After discarding the liquid, one pellet was frozen for later purification of plasmid DNA. To the second pellet was added 1.5 ml EtOH, and the pellet was resuspended by vortex mixing, and extraction was allowed to proceed in the dark for 15-30 min with occasional remixing. Insoluble materials were pelleted by centrifugation at maximum speed for 10 min in a microfuge. Absorption spectra of the supernatant fluids were recorded from 350-550 nm with a Perkin Elmer lambda six spectrophotometer.

#### Analysis of isolated clones

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Eight of the yellow colonies contained  $\beta$ -carotene indicating that a single gene product catalyzes both cyclizations required to form the two  $\beta$  endgroups of the symmetrical  $\beta$ -carotene from the symmetrical precursor lycopene. One of the yellow colonies contained a pigment with the spectrum characteristic of  $\delta$ -carotene, a monocyclic carotenoid with a single  $\epsilon$  endgroup. Unlike the  $\beta$  cyclase, this  $\epsilon$ -cyclase appears unable to carry out a second cyclization at the other end of the molecule.

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The observation that  $\epsilon$ -cyclase is unable to form two cyclic  $\epsilon$ -endgroups (e.g. the bicyclic  $\epsilon$ -carotene) illuminates the mechanism by which plants can coordinate and control the flow of substrate into carotenoids derived from  $\beta$ -carotene versus those derived from  $\alpha$ -carotene and also can prevent the formation of carotenoids with two  $\epsilon$  endgroups.

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The availability of the A. thaliana gene encoding the  $\epsilon$ -cyclase enables the directed manipulation of plant and algal species for modification of carotenoid content and

composition. Through inactivation of the  $\epsilon$ -cyclase, whether at the gene level by deletion of the gene or by insertional inactivation or by reduction of the amount of enzyme formed (by such as antisense technology), one may increase the formation of  $\beta$ -carotene and other pigments derived from it. Since vitamin A is derived only from carotenoids with  $\beta$  endgroups, an enhancement of the production of  $\beta$ -carotene versus  $\alpha$ -carotene may enhance nutritional value of crop plants. Reduction of carotenoids with  $\epsilon$ -endgroups may also be of value in modifying the color properties of crop plants and specific tissues of these plants. Alternatively, where production of  $\alpha$ -carotene, or pigments such as lutein that are derived from  $\alpha$ -carotene, is desirable, whether for the color properties, nutritional value or other reason, one may overexpress the  $\epsilon$ -cyclase or express it in specific tissues. Wherever agronomic value of a crop is related to pigmentation provided by carotenoid pigments the directed manipulation of expression of the  $\epsilon$ -cyclase gene and/or production of the enzyme may be of commercial value.

The predicted amino acid sequence of the A. thaliana  $\epsilon$ -cyclase enzyme was determined. A comparison of the amino acid sequences of the  $\beta$ - and  $\epsilon$ -cyclase enzymes of Arabidopsis thaliana (Fig. 13) as predicted by the DNA sequence of the respective cDNAs (Fig. 4 for the  $\epsilon$ -cyclase cDNA sequence), indicates that these two enzymes have many regions of sequence similarity, but they are only about 37% identical overall at the amino acid level. The degree of sequence identity at the DNA base level, only about 50%, is sufficiently low such that we and others have been unable to detect this gene by hybridization using the  $\beta$  cyclase as a probe in DNA gel blot experiments.

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Having now fully described the invention, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.

#### We claim:

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1. An isolated and/or purified nucleic acid sequence which encodes for a protein having lycopene  $\epsilon$ -cyclase enzyme activity and has an amino acid sequence which is at least 85% identical to one of SEQ ID NOS: 23 or 25-27.

- 5 2. The nucleic acid sequence of claim 1, wherein the protein has the amino acid sequence of one of SEQ ID NOS: 23 or 25-27.
  - 3. A vector comprising the nucleic acid sequence of claim 1, wherein the nucleic acid sequence is operably linked to a promoter.
  - 4. A host cell which contains the vector of claim 3.
- 5. The host cell of claim 4, wherein the host cell is selected from the group consisting of a bacterial cell, an algal cell, a yeast cell and a plant cell.
  - 6. The host cell of claim 4, wherein the host cell is a photosynthetic cell.
  - 7. An isolated and/or purified protein having lycopene  $\epsilon$ -cyclase enzyme activity and having an amino acid sequence which is at least 85% identical to one of SEQ ID NOS: 23 or 25-27.
  - 8. The protein of claim 7, wherein the protein has the amino acid sequence of one of SEQ ID NOS: 23 or 25-27.

#### AMENDED CLAIMS

[received by the International Bureau on 15 November 1999 (15.11.99); original claims 1,2,7 and 8 amended; remaining claims unchanged (1 page)]

- 1. An isolated and/or purified nucleic acid sequence which encodes for a protein having lycopene  $\epsilon$ -cyclase enzyme activity and has an amino acid sequence which is at least 85% identical to one of SEQ ID NOS: 23, 25 or 26.
- 5 2. The nucleic acid sequence of claim 1, wherein the protein has the amino acid sequence of one of SEQ ID NOS: 23, 25 or 26.
  - 3. A vector comprising the nucleic acid sequence of claim 1, wherein the nucleic acid sequence is operably linked to a promoter.
  - 4. A host cell which contains the vector of claim 3.

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- 5. The host cell of claim 4, wherein the host cell is selected from the group consisting of a bacterial cell, an algal cell, a yeast cell and a plant cell.
  - 6. The host cell of claim 4, wherein the host cell is a photosynthetic cell.
  - 7. An isolated and/or purified protein having lycopene ε-cyclase enzyme activity and having an amino acid sequence which is at least 85% identical to one of SEQ ID NOS: 23, 25 or 26.
  - 8. The protein of claim 7, wherein the protein has the amino acid sequence of one of SEQ ID NOS: 23, 25 or 26.

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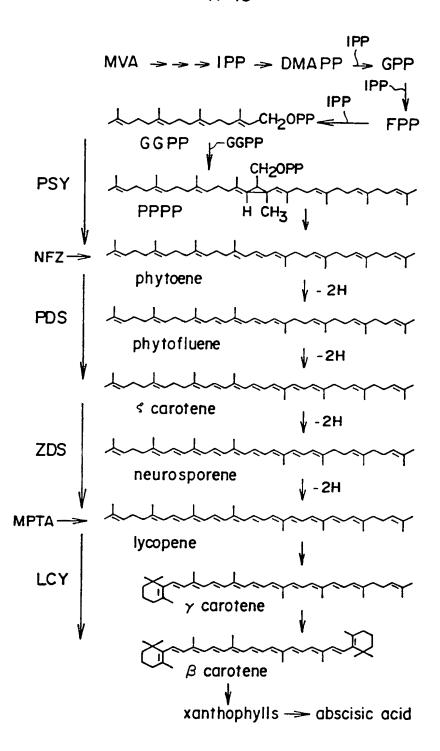


FIG. 1

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FIG. 4A

FIG. 4B

FIG. 4B

FIG. 11A

FIG. 11A

FIG. 11A

FIG. 13A
FIG. 13B
FIG. 13B
FIG. 14A
FIG. 14B

FIG. 22A
FIG. 22B

# FIG. 4A

Arabidopsis thaliana epsilon cyclase:

	acaaaaggaaataattag										attcctctttctgcttgctataccttgaca									48	
	gaa	agt	gctgtattcgaaattatttggaggaggaac																		
1	atg M	gag E	tgt:	gtt V	999 G	gct A	agg R	aat N	ttc F	gca A	gca A	atg M	gcg A	gtt V	tca S	aca T	ttt F	ccg P	tca <sup>.</sup> S	tgg W	168
21	agt S	tgt C	cga R	agg R	aaa K	ttt F	cca P	gtg V	gct V	aag K	aga R	tac Y	agc S	tat Y	agg R	aat N	att I	cgc R	ttc F	ggt G	228
41	ttg L	tgt C	agt S	gtc V	aga R	gct A	agc S	ggc G	ggc G	gga G	agt S	tcc S	ggt. G	agt S	gag K	agt: S	tgt C	gtag V	gcgg A	gtg V	288
61	aga R	gaa S	gat D	tto F	gct A	gac D	gaa E	gaa E	gat D	ttt F	gcg. V	aaa E	gct A	ggc G	ggt: G	tct S	gag. R	atto I	ctai L	ttt F	348
81	gtt V	caa Q	atg M	cag Q	cag Q	aac M	aaa K	gat D	atg M	gat D	gaa S	cag <b>Q</b>	tcta S	aag K	ctt	gtt V	gat D	aagt K	ttg( L	ct P	408
101	cct P	ata I	tca S	act I	ggt:	gate D	ggt G	gct: A	ttg L	gat D	cate K	gtg V	gtta V	act I	ggc G	tgt:	ggt. G	cct( P	gcto A	gt G	468
121	tta L	gcc A	ttg L	gct A	gca A	gaa <sup>.</sup> K	tca S	gct A	aag K	ctt L	gga G	tta L	aaa K	gtt V	gga <b>G</b>	ctc L	att I	ggto G	ccaç P	gat D	528
141	ctt L	cct P	ttt F	act T	aac M	aat M	tac Y	ggt G	gtt V	tgg M	gaa K	gate D	gaa <sup>.</sup> K	tta F	aato N	gato D	ctt L	999( G	ctgo L	caa G	588
161	aaa K	tgt. C	att I	gag <b>K</b>	cate K	gtt V	tgg W	aga R	gag S	act T	att	gcg( V	caco Y	ctg L	gate D	gate D	gac. D	aago K	ccta P	att I	648
181	acc T	att I	ggc G	cgt R	gct: A	tate Y	gga G	aga R	gtt V	agt S	cga R	cgt: R	ttg L	ctc	cato X	gag E	gag E	ctti L	ttga L	agg R	708
201	agg R	tgt C	gtc V	gag K	tca S	ggt G	gtc V	tcg S	tac Y	ctt L	agc S	tcg: S	aaa( <b>K</b>	gtt V	gaca D	agca S	ata I	acag T	gaag E	gct A	768
221	tgt S	gat D	ggc G	ctt L	aga X	ctt L	gtt <b>V</b>	gct: A	tgt C	gac D	gaca D	aata M	aacq M	gtc V	atto I	CCC	tgc C	aggo X	ctto L	gcc A	828
241	act T	gtt V	gct: A	tct S	gga G	gca A	gct A	tcg S	gga G	aag <b>K</b>	ctc	ttg( L	caat Q	tac Y	gaaq X	gtt V	ggt G	ggad G	cta P	aga R	888
	gto	tgt	gcg	caa	act	gca <sup>.</sup>	tac	ggc	gtg	gag	gtt	gag	gcg	gaa	aata	agto	cca	tato	gato	ca	948

# FIG. 4B

261	V C	\	/ (	) ]	Γ /	۹ ۱	Y (	G '	V	Χ	٧	X	٧	Χ	N	S	Р	Y	D	Р	
281	gatc D	aaa Q	atgo M	ytt V	ttc P	atgo M	gat D	tac Y	aga R	ngat D	tat Y	act T	aac M	gag X	aaa X	gtt V	cgg R	agc S	tta L	gaa X	1008
301	gctg A	agt K	tato Y	ccaa P	acg T	ttt F	ctg L	tac Y	gco A	atg M	cct P	atg M	aca T	aag K	tca S	aga R	ctc L	ttc F	ttc F	gag K	1068
321	gaga K	cat T	tgt: C	ttg L	gcc A	tca S	aaa K	gat D	gto V	catg M	ccc P	ttt F	gat D	ttg L	cta L	aaa K	acg T	aag K	ctc L	atg M	1128
341	ttaa I	igat P	tta V	gac G	aca G	cto S	gga L	att P	.cga N	aatt T	cta X	aag Q	jact K	tac N	gaa L	igag A	gag F	tgg G	tcc A	tat A	1188
361	atco I	ca P	gtt V	ggt. G	ggt G	tcc S	ttg L	cca P	aad <b>M</b>	cacc T	gaa X	icaa Q	iaag <b>K</b>	jaat N	ctc L	gcc A	ttt F	.ggt G	gct A	gcc A	1248
381	gcta A	igci S	atg M	gta V	cat M	ccc P	gca A	aca T	igg( G	ctat Y	tca S	gtt <b>V</b>	gtg V	jaga R	tct S	ttg L	rtct S	gaa X	gct A	cca P	1308
401	aaac K	ate Y	gca A	tca S	gtc V	atc I	gca A	gag <b>K</b>	jata I	acta L	aga R	igaa E	agag E	jact T	acc T	caaa K	cag Q	att I	aac N	agt S	1368
421	aata M	itt I	tca S	aga R	caa Q	gct A	tag W	gat D	ac T	ttta L	tgo W	gcca P	acca P	agaa E	aagg R	jaaa X	aga R	icag Q	jaga R	gca A	1428
441	ttc1 F	ttt F	ctc L	ttt F	ggt G	ctt L	gca A	cto	cag I	agtt V	caa Q	atto F	cgat D	taco T	cgaa X	1990 G	att I	aga R	agc S	ttc F	1488
461	ttc F	gt R	act T	ttc P	ttc F	cgc R	ctt L	CC2 P	aaa K	atgg W	ato M	gtgg W	gcaa Q	aggg	gtti F	tcta L	agga G	atca S	aca T	itta L	1548
481	aca <sup>r</sup>	tca S	igga G	gat D	cto	gtt V	cto	tt: F	tgc A	ttta L	tac Y	cato M	gtto P	egto V	cati I	ttca S	acca P	aaac M	caat M	ttg L	1608
501	aga. R	aaa K	ıggt G	cto	att I	aat N	cat W	cto	cat I	ctct S	gat D	tcc P	aaco T	cgga G	agca A	aaco T	cato M	gata I	aaaa <b>K</b>	acc T	1668
				•	atga	attt	tact	cta	cca	acto	tti	agg	ttt	gtg	tata	atai	tate	gccg	gatt	tat	1728
521	Y	_	K	•							_										1700
	-									gtgg											1788
	aga	ato	ctaa	agga	agt	gato	cgaa	aat	gga	igacç	ga.	aac	gaa	aag	aaa	aaa	atc	agto	ctti	tgtt	1848
	ccq	rtac	acta	agto	g																1868

#### FIG. 5

1 gctctttctc ctcctctt accgatttcc gactccgcct cccgaaatcc ttatccggat tctctccgtc tcttcgattt aaacgctttt ctgtctgtta 51 101 cgtcgtcgaa gaacggagac agaattctcc gattgagaac gatgagagac 151 cggagagcac gagctccaca aacgctatag acgctgagta tctggcgttg 201 cgtttggcgg agaaattgga gaggaagaaa tcggagaggt ccacttatct aatcgctgct atgttgtcga gctttggtat cacttctatg gctgttatgg 251 301 ctgtttacta cagattctct tggcaaatgg agggaggtga gatctcaatg 351 ttggaaatgt ttggtacatt tgctctctct gttggtgctg ctgttggtat 401 ggaattctgg gcaagatggg ctcatagagc tctgtggcac gcttctctat 451 ggaatatgca tgagtcacat cacaaaccaa gagaaggacc gtttgagcta aacgatgttt ttgctatagt gaacgctggt ccagcgattg gtctcctctc 501 551 ttatggattc ttcaataaag gactcgttcc tggtctctgc tttggcgccg 601 ggttaggcat aacggtgttt ggaatcgcct acatgtttgt ccacgatggt 651 ctcgtgcaca agcgtttccc tgtaggtccc atcgccgacg tcccttacct 701 ccgaaaggtc gccgccgctc accagctaca tcacacagac aagttcaatg 751 gtgtaccata tggactgttt cttggaccca aggaattgga agaagttgga 801 ggaaatgaag agttagataa ggagattagt cggagaatca aatcatacaa 851 aaaggcctcg ggctccgggt cgagttcgag ttcttgactt taaacaagtt 901 ttaaatccca aattctttt ttgtcttctg tcattatgat catcttaaga 951 cggtct

A.thal.	64 SFSS SSTDFRLRLP KSLSGFSPSL RFKRFSVCYV VEERRQNSPI ENDERPESTS STNAIDAEYL
A.thal. Alical. A.aurant. E.herb. E.ured. Consensus	ALRLAEKLER KKSERSTYLI AAMLSSFGIT SMAVMAVYYR FSWQMEGGEI SMLEMFGTFA LSVGAAVGME FWARWAHRAL.  MIOFL IVVATVLVME LTAYSVHRWI  ML.NSL IVVLSVIAME GIAAFTHRYI  ML.NSL IVILSVIAME GIAAFTHRYI  MLWIWNAL IVIVTVIGME VIAALAHKYI
	Predicted TM helix
A.thal. Alical. A.aurant. E.herb. E.ured. Consensus	WHASL.WNYH ESHHKPREGP FELNDVFAIV NAGPAIGLLS YGFFNKGLVP GLCFGAGLGI TVFGIAYMFV HDGLVHKRFP MHGPLGWGWH KSHHEEHDHA LEKNDLYGVV FAVLATILFT VGAYWMPVLW WIALGM TVYGLIYFIL HDGLVHORWP MHGPLGWGWH KSHHEEHDHA LEKNDLYGLV FAVIATVLFT VGWIWAPVLW WIALGM TVYGLIYFVL HDGLVHORWP MHG.WGWRWH ESHHTPRKGV FKLNDLFAVV FAGVAIALIA VGTAGVWPLQ WIGCGM TVYGLLYFLV HDGLVHORWP MHG.WGWGWH LSHHEPRKGA FEVNDLYAVV FAGVAIALIY LGSTGMWPLQ WIGCGM TAYGLLYFLV HDGLVHORWP H-1-WH -SHH-pr-g- fE-NDa-V -Aai-LGglg- Tv-GYv HDGLVH-R-P
	Predicted TM helix Predicted TM helix
A.thal. Alical. A.aurant. E.herb. E.ured. Consensus	VGPIADVPYL RKVAAAHQLH HTDKFNGV PYGLFLGPKE LEEVGGNEEL DKEISRRIKS YKKASGSGSS SS\$ FRYIPRRGYF RRLYQAHRLH HAVEGROHCV SFGFIYAPP. VDKLKODLKR SGVLRACDER PS* FRYIPRRGYA RRLYQAHRLH HAVEGROHCV SFGFIYAPP. VDKLKODLKM SGVLRACAGE RT* FHWIPRRGYL KRLYVAHRLH HAVRGREGCV SFGFIYARK. PADLOAILRE RHGRAPPKRDA AKDRAGGEDE PASGK*. FRYIPRRGYL KRLYMAHRMH HAVRGKEGCV SFGFIYARP. LSKLQATLRE RHGARAGA ARDAQGGEDE PASGK*IY] rAH-1H HVGp

1	ccacgggtco	geeteeeegt	ttttttccga	teegatetee	ggtgccgag
51	actcagctgt	ttgttcgcgc	tttctcagco	: gtcaccatga	Cogattotas
101	cgatgctgga	atggatgctg	ttcagagacg	actcatgttt	gaagacgaa
151	gcattctcgt	tgatgaaaat	aatcgtgtgg	tgggacatga	Cactaageae
201	aactgtcatc	tgatggaaaa	gattgaagct	gagaatttac	++caca
251	tttcagtgtg	tttttattca	actccaagta	tgagttgc++	stantagage
301	ggtcaaaaac	aaaggttact	ttcccactto	totoosca	ceccagcaac
351	agccatcctc	tttaccgtga	atcogagett	attraarra	cacttgttgc
401	tgtaagaaat	gccgcacaaa	ggaagetttt	castasaas	argracting
451		accagtegat			
501		ctgatgggaa			
551					
501		cgggatgtga			
551		cgtgagcagg			
701		atgaagctgt			
751		ttgatgaagt			
01		agacatgaaa			
51		tecettece			
01		aaaactggca			
51	aaaa	cttttgattc	atctctagtt	tagttttcat	cttaaaaaaa
	~~ <b>~</b>				

1	caccaatot	c tatttata			
51		e cyclette	t ttatttaat	c toccattgat	t tegeeteage
2.1	teteteget	c tttcgtctt	c tttttcttc	t ttccGATTT	CCCATCGTCC
101	TCTGTCATC	G ATTTCACCG	A GAAAGTTAC	C GAATTTTCGT	
151	GTACCGCTA	T GACAGATA	T AAACATCOT	G GTATGGATG	. delliciere
201	CCTCTCARC		- WWGWIGCI	G GTATGGATGC	TGTTCAGAGA
	COTCTCATG	r TTGAGGATG	A ATGCATTCT	r gttgatgaaa	CTGATCGTGT
251	TGTGGGGCA:	C GTCAGCAAG1	ATAATTGTC	A TCTGATGGAA	AATATTGAAG
301	CCAAGAATT	CCTGCACAGO	GCTTTTAGTO	TATTTTTATT	CAACTCGAAG
351	TATGAGTTG	TTCTCCAGCA	AAGGTCAAAC	ACAAAGGTTA	CGTTCCCTCT
401	AGTGTGGACT	AACACTTGTT	GCAGCCATCC	TCTTTACCGT	GAATCAGAGC
451				ATGCTGCACA	
501	CTCGATGAGC	TTGGTATTGT	AGCTGAAGAT	GTACCAGTCG	ATGAGTTCAG
551				TTCTGATGGC	
601				TGCGAGACGT	
651	CCAAACCCAG	ATCAACTACC	<b>***</b>	recovered	GAAGGITCAA
701		AIGAAGIAGC	TGAGATCAAG	TATGTGAGCC	GGGAAGAGCT
701	GAAGGAGCTG	GTGAAGAAAG	CAGATGCAGG	TGAGGAAGGT	TTGAAACTGT
751				TCTTGATGAA	
301	CATGTTGAGA	AAGGAACTTT	GGTTGAAGCT	ATAGACATGA	AAACCATCCA
351				TAAATCAATC	
01				TTTGGGATAT	
51					
		CCAAGCACCT	CAGGCAATAA	TAAAGTTTGC (	GGCGGC

1	CTCGGTAGCT	GGCCACAATC	GCTATTTGGA	ACCTGGCCCG	GCGGCAGTCC
51	GATGCCGCGA	TGCTTCGTTC	GTTGCTCAGA	GGCCTCACGC	ATATCCCCC
101	CGTGAACTCC	GCCCAGCAGC	CCAGCTGTGC	ACACGCGCGA	CTCCAGTTTA
151	AGCTCAGGAG	CATGCAGATG	ACGCTCATGC	AGCCCAGCAT	CTCAGCCAAT
201	CTGTCGCGCG	CCGAGGACCG	CACAGACCAC	ATGAGGGGTG	CAAGCACCTG
251	GGCAGGCGGG	CAGTCGCAGG	ATGAGCTGAT	GCTGAAGGAC	GAGTGCATCT
301	TGGTGGATGT	TGAGGACAAC	ATCACAGGCC	ATGCCAGCAA	GCTGGATGT
351	CACAAGTTCC	TACCACATCA	GCCTGCAGGC	CTGCTGCACC	GGGCCTTCTC
401	TGTGTTCCTG	TTTGACGATC	AGGGGCGACT	GCTGCTGCAA	CAGCGTGCAC
451	GCTCAAAAAT	CACCTTCCCA	AGTGTGTGGA	CGAACACCTG	CTGCAGCCAC
501	CCTTTACATG	GGCAGACCCC	AGATGAGGTG	GACCAACTAA	GCCAGGTGGC
551	CGACGGAACA	GTACCTGGCG	CAAAGGCTGC	TGCCATCCGC	AAGTTGGAGC
601	ACGAGCTGGG	GATACCAGCG	CACCAGCTGC	CGGCAAGCGC	GTTTCGCTTC
651	CTCACGCGTT	TGCACTACTG	TGCCGCGGAC	GTGCAGCCAG	CTGCGACACA
701	ATCAGCGCTC	TGGGGCGAGC	ACGAAATGGA	CTACATCTTG	TTCATCCGGG
751	CCAACGTCAC	CTTGGCGCCC	AACCCTGACG	AGGTGGACGA	AGTCAGGTAC
801	GTGACGCAAG	AGGAGCTGCG	GCAGATGATG	CAGCCGGACA	ACGGGCTGCA
851	ATGGTCGCCG	TGGTTTCGCA	TCATCGCCGC	GCGCTTCCTT	GAGCGTTGGT
901	GGGCTGACCT	GGACGCGGCC	CTAAACACTG	ACAAACACGA	GGATTGGGGA
951	ACGGTGCATC	ACATCAACGA	AGCGTGAAAG	CAGAAGCTGC	AGGATGTGAA
1001	GACACGTCAT	GGGGTGGAAT	TGCGTACTTG	GCAGCTTCGT	ATCTCCTTTT
1051	TCTGAGACTG	AACCTGCAGT	CAGGTCCCAC	AAGGTCAGGT	AAAATGGCTC
1101	GATAAAATGT	ACCGTCACTT	TTTGTCGCGT	ATACTGAACT	CCAAGAGGTC
1151	AAAAAAAA	AAAAA			

Ţ	CrcGGTAGCT	GGCCACAATC	GCTATTTGGA	ACCTGGCCCG	GCGGCAGTCC
51	GATGCCGCGA	TGCTTCGTTC	GTTGCTCAGA	GGCCTCACGC	ATATCCCGCG
101	CGTGAACTCC	GCCCAGCAGC	CCAGCTGTGC	ACACGCGCGA	CTCCAGTTTA
151	AGCTCAGGAG	CATGCAGCTG	CTTTCCGAGG	ACCGCACAGA	CCACATGAGG
201	GGTGCAAGCA	CCTGGGCAGG	CGGGCAGTCG	CAGGATGAGC	TGATGCTGAA
251	GGACGAGTGC	ATCTTGGTAG	ATGTTGAGGA	CAACATCACA	GGCCATGCCA
301	GCAAGCTGGA	GTGTCACAAG	TTCCTACCAC	ATCAGCCTGC	AGGCCTGCTG
351	CACCGGGCCT	TCTCTGTGTT	CCTGTTTGAC	GATCAGGGGC	GACTGCTGCT
401	GCAACAGCGT	GCACGCTCAA	AAATCACCTT	CCCAAGTGTG	TGGACGAACA
451	CCTGCTGCAG	CCACCCTTTA	CATGGGCAGA	CCCCAGATGA	GGTGGACCAA
501	CTAAGCCAGG	TGGCCGACGG	AACAGTACCT	GGCGCAAAGG	CTGCTGCCAT
551	CCGCAAGTTG	GAGCACGAGC	TGGGGATACC	AGCGCACCAG	CTGCCGGCAA
601	GCGCGTTTCG	CTTCCTCACG	CGTTTGCACT	ACTGTGCCGC	GGACGTGCAG
651	CCAGCTGCGA	CACAATCAGC	GCTCTGGGGC	GAGCACGAAA	TGGACTACAT
701	CTTGTTCATC	CGGGCCAACG	TCACCTTGGC	GCCCAACCCT	GACGAGGTGG
751 <sup>-</sup>	ACGAAGTCAG	GTACGTGACG	CAAGAGGAGC	TGCGGCAGAT	GATGCAGCCG
801	GACAACGGGC	TTCAATGGTC	GCCGTGGTTT	CGCATCATCG	CCGCGCGCTT
851	CCTTGAGCGT	TGGTGGGCTG	ACCTGGACGC	GGCCCTAAAC	ACTGACAAAC
901	ACGAGGATTG	GGGAACGGTG	CATCACATCA	ACGAAGCGTG	AAGGCAGAAG
951	CTGCAGGATG	TGAAGACACG	TCATGGGGTG	GAATTGCGTA	CTTGGCAGCT
1001	TCGTATCTCC	TTTTTCTGAG	ACTGAACCTG	CAGAGCTAGA	GTCAATGGTG
1051	CATCATATTC	ATCGTCTCTC	TTTTGTTTTA	GACTAATCTG	TAGCTAGAGT
1101	CACTGATGAA	TCCTTTACAA	CTTTCAAAAA	AAAAA	

# FIG. IIA

HPO4 HPO5 ATDP7 C.brew. ATOP5 S.cerev.	1 MLRSLLRGLT MLRSLLRGLT MSVSSLFNLP MS.SSMLNFT MTADNNSM	HIPRVNSAQQ .LIRLRSLA. .ASRIVSLPL .TGPPPRFFP	PSCAHARLÓF LSSSFSSFRF LSSPPSRVHL IRSPVPRTQL	KLRSMOLL AHRPLSSIS. PLCFFSPISL FVRAFSAV	PRKLPNFRAF TQRFSAKLTF
	SEDRTDHMRG SGTA.MTD SSQATT.MGE T.MTD	ASTWAGGOSO ASTWAGGOSO TKDAGMDAVO VVDAGMDAVO SNDAGMDAVO ETCFSGHDEE	DELMLKDECI RRLMFEDECI RRLMFEDECI RRLMFEDECI	LVDVEDNITG LVDVEDNITG LVDETDRVVG LVDENDKVVG LVDENNRVVG VLDWDDNAIG	100 HASKLECHKF HASKLECHKF HVSKYNCHLM HESKYNCHLM HDTKYNCHLM AGTKKVCHLM
	LPHOPAGLLH ENIEAKNLLH ENIESENLLH EKIEAENLLH	RAFSVFLFDD RAFSVFLFNS RAFSVFLFNS RAFSVFLFNS RAFSVFLFNS RAFSVFIFNE	QGRLLLQQRA QGRLLLQQRA KYELLLQQRS KYELLLQQRS KYELLLQQRS QGELLLQQRA	RSKITFPSVW NTKVTFPLVW ATKVTFPLVW KTKVTFPLVW	150 TNTCCSHPLH TNTCCSHPLH TNTCCSHPLY TNTCCSHPLY TNTCCSHPLY TNTCCSHPLC
	151 GQTPDEVDQL GQTPDEVDQL RE RE IDDELGL		AKAAAIRKLE AKAAAIRKLE VRNAAQRKLL VRNAAQRKLL VRNAAQRKLF AITAAVRKLD	HELGIPAHÒL DELGIVAEDV DELGIPAEDL DELGIVAEDV	200 PA.SAFRFLT PA.SAFRFLT PV.DEFTPLG PV.DQFIPLS PV.DEFTPLG KTRGKFHFLN
	RLHYCAADVQ RMLY RILY	PAATQSALWG PAATQSALWG .KAPSDGKWG .KAPSDGKWG .KAPSDGKWG .MAPSNEPWG	EHEMDYILFI EHELDYLLFI EHELDYLLFI EHEVDYLLFI	RANVTLRANVTLVRDVKVIRDVNLVRDVKL KINAKENLTV	APNPDEVDEV QPNPDEVAEI DPNPDEVAEV OPNPDEVAEI

## FIG. IIB

300
RYVTQEELRQ MMQ...PDN GLQWSPWFRI IAARFLERWW ADLDAALNTD
RYVTQEELRQ MMQ...PDN GLQWSPWFRI IAARFLERWW ADLDAALNTD
KYVSREELKE LVKKADAGEE GLKLSPWFRL VVDNFLMKWW DHVEKGTLVE
KYMNRDDLKE LLRKADAEEE GVKLSPWFRL VVDNFLFKWW DHVEKGSLKD
KYVSREELKE LVKKADAGDE AVKLSPWFRL VVDNFLMKWW DHVEKGTITE
KWVSPNDLKT MF....ADP SYKFTPWFKI ICENYLFNWW EQLDDLSEVE

301
KHEDWGTVHH INEA\*
KHEDWGTVHH INEA\*
A.IDMKTIHK L\*
A.ADMKTIHK L\*
A.ADMKTIHK L\*
A.ADMKTIHK L\*
NDRQ...IHR ML\*

## F I G. 12

ccaaaaacaa ctcaaatctc ctccgtcgct cttactccgc catgggtgac 1 gacteeggea tggatgetgt teagegaegt eteatgtttg acgatgaatg 51 cattttggtg gatgagtgtg acaatgtggt gggacatgat accaaataca 101 attgtcactt gatggagaag attgaaacag gtaaaatgct gcacagagca 151 ttcagcgttt ttctattcaa ttcaaaatac gagttacttc ttcagcaacg 201 gtctgcaacc aaggtgacat ttcctttagt atggaccaac acctgttgca 251 301 gecatecact ctacagagaa teegagettg tteeegaaac geetgagaga 351 401 451 501 551 601 xxxxxxxxx xxxxxxxx xxxxxxxxx tcatgtgcaa aagggtacac 651 701 tcactgaatg caatttgata tgaaaaccat acacaagctg atatagaaac acacceteaa eegaaaagea ageetaataa ttegggttgg gtegggteta 751 ccatcaattg tttttttttt ttaacaactt ttaatctcta tttgagcatg 801 ttgattcttg tcttttgtgt gtaagatttt gggtttcgtt tcagttgtaa 851 901 taatgaacca ttgatggttt gcaatttcaa gttcctatcg acatgtagtg 951 atctaaaaa

# F1G. 13 A

1 MECVGARNFA AMAVSTFPSW SCRRKFPVVK RYSYRNÍRFG LCSVRASGGG SSGSESCVAV REDFÄDEXDF	Cyanobacterial enzyme begins 71 VKSsALLa LVPETKKKNL DFELPmYDp. S.Kg-VV DLAVVGGGPA GLAVAQQVSE AGLSVcSIDp VK4-SsALLa LVPETKKKNL DFELPmYDp. S.Kg-VV DLAVVGGGPA GLAVAQQVSE AGLSVCSIDp VKAGGSEIL. FVQMQQNKDM DEQSKLVDKL PPISIGDGAL DHVVIGCGPA GLALAAESAK LGLKVGLIGP VKSLV DDS fg-GPA GLA-AGL-VI-P	Possible subunit interaction domain Dinucleotide-binding signature	210 PKLIWPNN YGVWVDEFEA MDLLDCLDaT WSGa-VYiDd -t-KDL-RPY GRVNRKQLKS KMMQKCI-NG DLPFTNN YGVWEDEFND LGLQKCIEHV WRETIVYLDD DKPITIGRAY GRVSRRLLHE ELLRRCVESG PNN YGVW-DEFLC WVY-DDR-Y GRV-RL	Conserved region #1	280 VKFHGaKVik ViHE.E-kSm liCnDG-tIQ AtVVLDATGF SRLVQYDK PYnPGY.QVA YGILAEVeeH VSYLSSKVDS ITKASDGLRL VACDDNNVIP CRLATVASGA ASGKLLQYEV GGPRVCVQTA YGVEVEVENS VKVQ-A YGgv
Plant <i>beta</i> A.t.epsilon Consensus	Plant <i>beta</i> A.t.epsilon Consensus	Pos	Plant <i>beta</i> A.t.epsilon Consensus		Plant beta A.t.epsilon Consensus

420 LASe L--350 JERMVARL (TKLMLRL TM helix  $\infty$ 480 AFFDLaPryw TFFRLPKWW Predicted RTLAAAPVVA RSLSEAPKYA LVARPGLrmd LASKDVMPFD R-L--AP--A #3 VHPSTGYMVA VHPATGYSVV VHP-TGY-V-OLDATRRFFD OTEGIRSFFR Conserved region #4 Conserved region WGiGGTAGM NLAFGAAASM N IMTK.GT-P LINHLISDP ----G---H CFGMDILLKL LFGLALIVQF -FG-----#2 Conserved region FShasntsr- I FVISPNNLRK F----R-GGPLPV1POR GGSLPNTEOK GG-LP---0--eLKERNs-i .KVRSLEAEY -ER-ROR-F SaeVWKDLWP SRQAWDTLWP S---W--LWP PELivFGLSL GDLVLFALYM Predicted TM helix DWRDSHL-NN DYRDY..TNE D-RD----N-Conserved region #2 EEDEHCVIPM (EEEWSVIPV -E-E---IP-281 PFD--KMV fM PYDPDQMVFM P-D---MVFM 481 HGFLSSRLFL QGFLGSTLTS -GFL-S-L---HLGIKVKSI DTLGTRILKT --LGI-----Plant beta A.t.epsilon Plant beta A. t.epsilon Plant beta A.t.epsilon Plant beta A.t.epsilon Consensus Consensus Consensus Consensus

#### F1G. 14A

Adonis palaestina ε-cyclase cDNA #5 Length: 1898

```
aaaggagtgt tctattaatg ttactgtcgc attcttgcaa cacttatatt
    1
       caaactccat tttcttcttt tctcttcaaa acaacaaact aatgtgagca
   51
  101
       gagtatctgg ctatggaact acttggtgtt cgcaacctca tctcttcttg
      ccctgtgtgg acttttggaa caagaaacct tagtagttca aaactagctt
  151
       ataacataca tcgatatggt tcttcttgta gagtagattt tcaagtgaga
 201
 251
      gctgatggtg gaagcgggag tagaagttct gttgcttata aagagggttt
 301
      tgtggatgaa gaggatttta tcaaagctgg tggttctgag cttttgtttg
 351
      tccaaatgca gcaaacaaag tctatggaga aacaggccaa gctcgccgat
      aagttgccac caataccttt tggagaatcc gtgatggact tggttgtaat
 401
 451
      aggttgtgga cctgctggtc tttcactggc tgcagaagct gctaagctag
      ggttgaaagt tggccttatt ggtcctgatc ttccttttac aaataattat
 501
      ggtgtgtggg aagacgagtt caaagatctt ggacttgaac gttgtatcga
 551
 601
      gcatgcttgg aaggacacca tcgtatatct tgataatgat gctcctgtcc
 651
      ttattggtcg tgcatatgga cgagttagtc gacatttgct acatgaggag
      ttgctgaaaa ggtgtgtgga gtcaggtgta tcatatctgg attctaaagt
 701
      ggaaaggatc actgaagctg gtgatggcca tagccttgta gtttgtgaaa
 751
      atgagatett tatecettge aggettgeta etgttgeate tggageaget
 801
 851
      tcagggaaac ttttggagta tgaagtaggt ggccctcgtg tttgtgtcca
 901
      aaccgcttat ggggtggagg ttgaggtgga gaacaatcca tacgatccca
 951
      acttaatggt attcatggac tacagagact atatgcaaca gaaattacag
1001
      tgctcggaag aagaatatcc aacatttctC tatgtcatgc ccatgtcgcc
      aacaagactt ttttttgagg aaacctgttt ggcctcaaaa gatgccatgc
1051
1101
      cattcgatct actgaagaga aaactgatgt cacgattgaa gactctgggt
1151
      atccaagtta caaaagttta tgaagaggaa tggtcatata ttcctgttgg
      tggttcttta ccaaacacag agcaaaagaa cctagcattt ggtgctgcag
1201
1251
      caagcatggt gcatccagca acaggctatt cggttgtacg gtcactgtca
      gaagctccaa aatatgcttc tgtaattgca aagattttga agcaagataa
1301
1351
      ctctgcgtat gtggtttctg gacaaagtag tgcagtaaac atttcaatgc
      aagcatggag cagtctttgg ccaaaggagc gaaaacgtca aagagcatTc
1401
      tttcttttTg gattagagct tattgtgcag ctagatattg aagcaaccag
1451
1501
     aacattettt agaacettet teegettgee aacttggatg tggtggggtt
     tccttgggtc ttcactatca tctttcgatc tcgtcttgtt ttccatgtac
1551
     atgtttgttt tggcgccaaa cagcatgagg atgtcacttg tgagacattt
1601
     gctttcagat ccttctggtg cagttatggt aagagcttac ctcgaaaggt
1651
1701
     agteteatet attattaaae tetagtgitt caccaaataa atgaggatee
1751
     ticgaatgtg tatatgatca tctctatgta tatcctgtac tctaatctca
     taaagtaaat gccgggtttg atattgttgt gtcaaaccgg ccaatgatat
1801
     aaagtaaatt tattgataca aaagtagttt ttttccttaa aaaaaaaa
1851
```



## FIG. 14B

Adonis palaestina E-cyclase #5 predicted polypeptide TRANSLATE from: 113 to: 1702 Length: 529 amino acids

1	MELL CVDNLT	SSCDVAITEGT	DNII CCCVI AV	NIHRYGSSCR	MUEUMDADCC
1					
51	SGSRSSVAYK	EGFVDEEDFI	KAGGSELLFV	QMQQTKSMEK	QAKLADKLPP
101	IPFGESVMDL	VVIGCGPAGL	SLAAEAAKLG	LKVGLIGPDL	PFTNNYGVWE
151	DEFKDLGLER	CIEHAWKDTI	VYLDNDAPVL	<b>IGRAYGRVSR</b>	HLLHEELLKR
201	CVESGVSYLD	SKVERITEAG	<b>DGHSLVVCEN</b>	EIFIPCRLAT	VASGAASGKL
251	LEYEVGGPRV	CVQTAYGVEV	<b>EVENNPYDPN</b>	LMVFMDYRDY	MOOKLOCSEE
301	EYPTFLYVMP	MSPTRLFFEE	TCLASKDAMP	FDLLKRKLMS	RLKTLGIQVT
351	KVYEEEWSYI	<b>PVGGSLPNTE</b>	QKNLAFGAAA	<b>SMVHPATGYS</b>	VVRSLSEAPK
401	YASVIAKILK	QDNSAYVVSG	QSSAVNISMQ	AWSSLWPKER	KRQRAFFLFG
451	LELIVQLDIE	ATRTFFRTFF	RLPTWMWWGF	LGSSLSSFDL	VLFSMYMFVL
501	APNSMRMSLV	RHLLSDPSGA	VMVRAYLER*		



#### FIG. 15A

DNA sequence of potato cDNA (GenBank R27545) obtained from Nicholas J. Provart

```
potato.seg Length: 1378 August 2, 1996 13:06 Type: N Check: 605 ...
     tagcggnnnn naggatgagt tcaaagatct tggtcttcaa gcctgcattg
 51
     aacatgtttg gcgggatacc attgtatatc ttgatgatga tgatcctatt
 101
     cttattggcc gtgcctatgg aagagttagt cgccatttac tgcacgagga
     gttactcaaa aggtgtgtgg aggcaggtgt tttgtatcta aactcgaaag
 151
     tggataggat tgttgaggcc acaaatggcc acagtcttgt agagtgcgag
 201
     ggtgatgttg tgattccctg caggtttgtg actgttgcat cgggagcagc
 251
 301
     ctcggggaaa ttcttgcagt atgagttggg aggtcctaga gtttctgttc
 351
     aaacagctta tggagtggaa gttgaggtcg ataacaatcc atttgacccg
 401
     agcctgatgg ttttcatgga ttatagagac tatgtcagac acgacgctca
451
     atctttagaa gctaaatatc caacatttct ctatgccatg cccatgtctc
501
     caacacgagt ctttttcgag gaaacttgtt tggcttcaaa agatgcaatg
551
     ccattcgatc tgttaaagaa aaaattgatg ttacgattga acaccctcgg
601
     tgtaagaatt aaagaaattt atgaggagga atggtcttac ataccagttg
651
     gaggatettt gecaaataca gaacaaaaaa caettgeatt tggtgetget
 701
     gctagcatgg ttcatccagc cacaggttat tcagtcgtca gatcactgtc
 751
     tgaagctcca aaatgcgcct tcgtgcttgc aaatatatta cgacaaaatc
801
     atagcaagaa tatgcttact agttcaagta ccccgagtat ttcaactcaa
851
     qcttqqaaca ctctttggcc acaagaacga aaacgacaaa gatcgttttt
 901
     cctatttgga ctggctctga tattgcagct ggatattgag gggataaggt
 951
     catttttccq cqcgttcttc cgtgtgccaa aatggatgtg gcagggattt
1001
     cttggttcaa gtctttcttn agcagacctc atgttatttg ccttctacat
1051
     qtttattatt qcaccaaatq acatqaqaaq aqqcttaatc aqacatcttt
1101
     tatctgatcc tactggtgca acattgataa gaacttatct tacattttag
1151
     agtaaattcc tcctacaata gttgttgaan nagaggcctc attacttcag
1201
     attcataaca gaaatcgcgg tctctcgagg ccttgtatat aacattttca
     ctaggttaat attgcttgaa taagttgcac agtttcagtt tttgtatctg
1251
     cttcttttt gtccaagatc atgtattgan ccaatttata tacattgcca
1301
1351
     qtatatataa attttataaa aaaaaaaa
```

poteps.pep Length: 378 TRANSLATE from: 14 to: 1147

- DEFKDLGLQÄ CIEHVWRDTI VYLDDDDPIL IGRAYGRVSR HLLHEELLKR CVEAGVLYLN SKVDRIVEAT NGHSLVECEG DVVIPCRFVT VASGAASGKF LQYELGGPRV SVQTAYGVEV EVDNNPFDPS LMVFMDYRDY VRHDAQSLEA KYPTFLYAMP MSPTRVFFEE TCLASKDAMP FDLLKKKLML RLNTLGVRIK EIYEEEWSYI PYGGSLPNTE QKTLAFGAAA SMVHPATGYS VVRSLSEAPK CAFVLANILR QNHSKNMLTS SSTPSISTQA WNTLWPQERK RQRSFFLFGL ALILQLDIEG IRSFFRAFFR VPKWMWQGFL GSSLSXADLM LFAFYMFIIA
- 351 PNDMRRGLIR HLLSDPTGAT LIRTYLTF\*

#### FIG. 15B

Chimeric lettuce/potato lycopene  $\epsilon$ -cyclase: converts lycopene to  $\delta$ -carotene, the lettuce cDNA converts lycopene to  $\epsilon$ -carotene and the potato cDNA does not produce an active enzyme

(amino acids in lower case are from lettuce and those in uppercase are from the potato cDNA; an  $Ava\Pi$  site in common to the two cDNAs was used to construct the chimera)

mecfgarnmt atmavftcpt ftdcnirhkf sllkqrrftn lsassslrqi kcsaksdrcv vdkqgisvac eedyvkaggs elffvqmqrt ksmesqskls 51 eklaqipign cildlvvigc gpaglalaae saklglnvgl igpdlpftnn 101 ygvwqdefig lglegciehs wkdtlvyldd adpirigray grvhrdllhe 151 ellrrcvesg vsylsskver iteapngysl iecegnitip crlatvasga 201 asgkfleyel gGPRVSVQTA YGVEVEVDNN PFDPSLMVFM DYRDYVRHDA 251 OSLEAKYPTF LYAMPMSPTR VFFEETCLAS KDAMPFDLLK KKLMLRLNTL 301 351 GVRIKEIYEE EWSYIPVGGS LPNTEQKTLA FGAAASMVHP ATGYSVVRSL 401 SEAPKCAFVL ANILRONHSK NMLTSSSTPS ISTQAWNTLW PQERKRQRSF 451 FLFGLALILQ LDIEGÌRSFF RAFFRVPKWM WQGFLGSSLS XADLMLFAFY MFIIAPNDMR RGLIRHLLSD PTGATLIRTY LTF\* 501

# F1G.16

	mparison of Arabidopsis E-cyclase x potato E-cyclase (partial) m62.cmp Gap Weight: 12 Average Match: 2.912 Length Weight: 4 Average Mismatch: -2.003 Quality: 1485 Length: 529 Ratio: 3.929 Gaps: 1
Match	Ratio: 3.929 Gaps: 1 Percent Similarity: 79.893 Percent Identity: 76.139 display thresholds for the alignment(s):   = IDENTITY := 2 . = 1
151 1	EDEFNDLGLOKCIEHVWRETIVYLDDDKPITIGRAYGRVSRRLLHEELLR 200
201	RCVESGVSYLSSKVDSITEASDGLRLVACDDNNVIPCRLATVASGAASGK 250
50	RCVEAGVLYLNSKVDRIVEATNGHSLVECEGDVVIPCRFVTVASGAASGK 99
251	LLQYEVGGPRVCVQTAYGVEVEVENSPYDPDQMVFMDYRDYTNEKVRSLE 300
100	.
301	AEYPTFLYAMPMTKSRLFFEETCLASKDVMPFDLLKTKLMLRLDTLGIRI 350
150	.        .
351	LKTYEEEWSYIPVGGSLPNTEOKNLAFGAAASMVHPATGYSVVRSLSEAP 400
200	.
401	KYASVIAEILREETTKQINSNISRQAWDTLWPPERKRQRAFFLFG 445
250	:      : .
446	LALIVOFDTEGIRSFFRTFFRLPKWMWQGFLGSTLTSGDLVLFALYMFVI 495
300	.             .       . .   .      :  LALILQLDIEGIRSFFRAFFRVPKWMWQGFLGSSLSXADLMLFAFYMFII 349
496	SPNNLRKGLINHLISDPTGATMIKTYLKV 524
350	.  .: :      :      : :    APNDMRRGLIRHLLSDPTGATLIRTYLTF 378



#### FIG. 17A

#### Adonis palaestina Ipil attcatcttc agcagcgctg tcgtactctt tctatatctt cttccatcac 51 taacagtagt cgccgacggt tgaatcggct attcgcctca acgtcaacta tgggtgaagt cactgatgct ggaatggatg ctgttcagaa gcggctcatg 101 ttcgacgacg aatgtatttt ggtggatgag aatgacaagg tcgtcgggca 151 tgattccaaa tacaactgtc atttgatgga aaagatagag gcagaaaatt 201 251 tgcttcacag agccttcagt gttttcttgt tcaactcaaa atatgaattg 301 cttcttcagc aacgatccgc cacaaaggta acattcccgc tcgtatggac aaacacatgt tgcagtcatc ctctctttcg tgattccgag ctcatagaag 351 401 aaaattatct cggtgtacga aacgctgcac aaagaaagct tttagacgag ctaggcattc cagctgaaga tgtcccagtt gatgaattta ctcctcttgg 451 tcqcattctt tacaaagctc catctgacgg caaatgggga gagcacgaat 501 tggactatct cctatttatt gtccgagatg tgaaatacga tccaaaccca 551 gatgaagttg ctgatgctaa gtatgttaat cgcgaggagt tgagagagat 601 actgagaaaa gctgatgctg gtgaagaggg actcaagttg tctccttggt 651 ttagattggt tgttgataac tttttgttca agtggtggga tcatgtagag 701 cagggtacga ttaaggaagt tgctgacatg aaaactatcc acaagttgac 751 ttaagaggac ttctctcctc tgttctacta tttgtttttt gctacaataa 801 gtgggtggtg ataagcagtt titctgtttt ctttaattta tggcttttga 851 atttgcctcg atgttgaact tgtaacatat ttagacaaat atgagacctt 901 gtaagttgaa tttgaggctg aatttatatt tttgggaaca taataatqtt 951 1001 aa

## FIG. 17B

Adonis palaestina Ipi2 ttttaaagct ctttcgctcc accaccatca aagccagcca aatttctctg tacaaaagtt aaaaacaccg ctttgggctt tggcccctcc atatcggaat 101 ccttgtttac gatacgcatc taaaccagta attctcggtt ttaatttgtt tcctaaatta ggcccctttc cggaatcccg agaattatgt cgtcgatcag 151 gattaatcct ttatatagta tcttctccac caccactaaa acattatcag 201 cttcgtgttc ttctcccgct gttcatcttc agcagcgttg tcgtactctt 251 tctatttctt cttccatcac taacagtcct cgccgagggt tgaatcggct 301 gttcgcctca acgtcgacta tgggtgaagt cgctgatgct ggtatggatg 351 ccgtccagaa gcggcttatg ttcgacgatg aatgtatitt ggtggatgag 401 aatgacaagg tcgtcggaca tgattccaaa tacaactgtc atttgatgga 451 aaagatagag gcagaaaact tgcttcacag agccttcagt gttttcttat 501 tcaactcaaa atacgagttg cttcttcagc aacgatctgc aacgaaggta 551 acattecege tegtatggac aaacacetgt tgeagecate ecetetteeg 601 tgattccgaa ctcatagaag aaaattttct cggggtacga aacgctgcac 651 aaaggaagct tttagacgag ctaggcattc cagctgaaga cgtaccagtt 701 gatgaattca ctcctcttgg tcgcattctt tacaaagctc catctgacgg 751 aaaatgggga gagcacgaac tggactatct tctgtttatt gtccgagatg 801 tgaaatacga tccaaaccca gatgaagttg ctgacgctaa gtacgttaat 851 cgcgaggagt tgaaagagat actgagaaaa gctgatgcag gtgaagaggg 901 aataaagtig teteetiggt ttagaitggt igtggaiaac itittgitea 951 agtggtggga tcatgtagag gaggggaaga ttaaggacgt cgccgacatg 1001 aaaactatcc acaagttgac ttaagagaaa gtctcttaag ttctactatt 1051 tggtttttgc ttcaataagt ggatggtgat gagcagtttt tatgcttcct 1101 ttaattttgg cttttcaatt tgctttatgt gttgaacttg taacatattt 1151 agtcaaatat gagaccttgt gagttgaatt tgaggttata tttatagttt 1201 tgggaacata aaaaaaaaaa 1251



## FIG. 18A

Haematococcus pluvialis Ipil ctcggtagct ggccacaatc gctatttgga acctggcccg gcggcagtcc gatgccgcga tgcttcgttc gttgctcaga ggcctcacgc atatcccccg 51 cgtgaactcc gcccagcagc ccagctgtgc acacgcgcga ctccagttta 101 agctcaggag catgcagatg acgctcatgc agcccagcat ctcagccaat 151 ctgtcgcgcg ccgaggaccg cacagaccac atgaggggtg caagcacctg 201 ggcaggcggg cagtcgcagg atgagctgat gctgaaggac gagtgcatct 251 tggtggatgt tgaggacaac atcacaggcc atgccagcaa gctggagtgt 301 cacaagttcc taccacatca gcctgcaggc ctgctgcacc gggccttctc 351 tgtgttcctg tttgacgatc aggggcgact gctgctgcaa cagcgtgcac 401 gctcaaaaat caccttccca agtgtgtgga cgaacacctg ctgcagccac 451 cctttacatg ggcagacccc agatgaggtg gaccaactaa gccaggtggc 501 cgacggaaca gtacctggcg caaaggctgc tgccatccgc aagttggagc 551 acgagetggg gataccageg caccagetge eggeaagege gtttegette 601 ctcacgcgtt tgcactactg tgccgcggac gtgcagccag ctgcgacaca 651 atcagcgctc tggggcgagc acgaaatgga ctacatcttg ttcatccggg 701 ccaacgtcac cttggcgccc aaccctgacg aggtggacga agtcaggtac 751 gtgacgcaag aggagctgcg gcagatgatg cagccggaca acgggctgca 801 atggtcgccg tggtttcgca tcatcgccgc gcgcttcctt gagcgttggt 851 gggctgacct ggacgcggcc ctaaacactg acaaacacga ggattgggga 901 acggtgcatc acatcaacga agcgtgaaag cagaagctgc aggatgtgaa 951 gacacgtcat ggggtggaat tgcgtacttg gcagcttcgt atctcctttt 1001 tctgagactg aacctgcagt caggtcccac aaggtcaggt aaaatggctc 1051 gataaaatgt accgtcactt tttgtcgcgt atactgaact ccaagaggtc 1101 aaaaaaaaa aaaaa 1151

#### FIG. 18B

Haematococcus pluvialis Ipi2 tggaacctgg cccggcggca gtccgatgcc gcgatgcttc gttcgttgct cagaggeete acgeatatee egegegtgaa eteegeecag cageecaget 51 gtgcacacge gegactecag titaagetea ggagcatgea getgettgee 101 gaggaccgca cagaccacat gaggggtgca agcacctggg caggcgggca 151 gtcgcaggat gagctgatgc tgaaggacga gtgcatctta gtggatgctg 201 acgacaacat cacaggccat gccagcaagc tggagtgcca caaattccta 251 ccacatcage etgeaggeet getgeacegg geettetetg tgtteetgtt 301 tgacgaccag gggcgactgc tgctgcaaca gcgtgcacgc tcaaaaatca 351 ccttcccaag tgtgtggacg aacacctgct gcagccaccc tctacatggg 401 cagaccccag atgaggtgga ccaactaagc caggtggccg acggcacagt 451 acctggcgca aaagctgctg ccatccgcaa gttggagcac gagctgggga 501 taccagegea ccagetgeeg geaagegegt ttegetteet caegegittg 551 cactactgtg ccgcggacgt gcagccggct gcgacacaat cagcgctctg 601 gggcgagcac gagatggact acatettatt catecgggcc aacgteacet 651 tggcgcccaa ccctgacgag gtggacgaag tcaggtacgt gacgcaagag 701 gagctgcggc agatgatgca gccggacaac gggttgcaat ggtcgccgtg 751 gtttcgcatc atcgccgcgc gcttccttga gcgttggtgg gctgacctgg 801 acgcggcct aaacactgac aaacacgagg attggggaac ggtgcatcac 851 atcaacgaag cgtgaaggca gaagctgcag gatgtgaaga cacgtcatgg 901 ggtggaattg cgtacttggc agcttcgtat ctcctttttc tgagactgaa 951 cctgcagagc tagagtcaat ggtgcatcat attcatcgtc tctcttttgt 1001 tttagactaa tctgtagcta gagtcactga tgaatccttt acaactttca 1051 1101 aaaaaaaa

## FIG. 19A

Lactuca sativa Ipil tgccaaaatg ttgaaatttc ccccttttaa aaccattgct accatgatct cttctccată ttcttccttc ttgctgcctc ggaaatcttc tttccctcca atgccgtctc tcgcagccgc tagtgttttc ctccaccctc tttcgtctgc cgctatgggc gattccagca tggatgctgt ccagcgacgt ctcatgttcg 101 151 201 atgacgaatg cattttggtg gatgagaatg acaaagtggt tggccatgat actaaataca attgtcattt gatggagaag attgaaaagg gaaatatgct 251 301 acacagagca ttcagtgtgt tcttgttcaa ctcgaaatat gaattactcc ttcagcaacg ttctgcaacc aaggtgactt tccctttggt atggacaaac 351 401 acgtgttgcă gccatccact atacagggag agtgagcfta ttgacgaaaa cgcccttggg gtgaggaatg ctgcacagag gaagctcctg gatgaactcg gcatcctgg agcagatgtt ccggttgatg agttcactcc attgggtcgc 451 501 attctataca aggccgcatc ggatggaaag tggggagaac atgaacttga ttacctgctg tttatggtac gtgatgttgg tttggatccg aacccagatg 551 601 aagtgaaaga totaaaatat otgaaccooo aagaoctgaa ggaattoota 651 aggaaggcgg atgctggtga agagggtgtg aagctgtccc cgtggttcaa attgattgtc gataatttct tgtttcagtg gtgggatcga ctccataagg 701 751 gaaccctaac cgaagctatt gatatgaaaa caatccacaa actcacataa 801 ăaacactaca ctagtaggag ăgaggăttat atgagatatt tgttatatgt gaaattgaaa ttcagatgaa tgcttgtatt tatttctatt tggacaaact 851 901 tcaacttctt tttgctacct tatcagaaaa aaaaa 951

#### FIG. 19B

Lactuca sativa Ipi2 tattcgcttc aaaatctctt ccattaactg ctcaaatctc caccttcgcc ggtcttaatc tccgccggcg cactttcacc accataaccg ccgccatggg tgacgattcc ggcatggacg ctgtccagag acgtctcatg tttgatgatg aatgcatttt ggttgatgaa aatgacaatg ttcttgggca tgataccaaa 101 151 tacaattgtc acttgatgga gaagattgag aaagafaatt tgcttcatag 201 agcattcagt gtattttat tcaattcaaa atacgaatta ctccttcagc 251 aaaggtcaga aaccaaggtg acatttcctt tggtatggac aaacacctgt tgcagccatc cactatacag agaatcggag ttaattcccg aaaatgccct 301 351 tggggtcaga aatgctgcac agaggaagct tctagatgaa ctcggtatcc ctgctgaaga tgttccagtt gatgagttca caactttagg tcgcatgttg 401 451 tačaağgete cătetgatgg aaaatggggt gaacatgaag ttgattacet 501 actettecte gtgcgtgacg ttgccgtgaa cccaaaccet gatgaggtgg 551 cggacattag ătăcğtgaac caagaagagt taaaagagtt actaaggaag 601 gcggatgcgg gtgaggggg tttgaaattg tccccatggt ttaggctagt 651 701 ğgtggacaac ttcttgttca aatggtggga tcatgtccaa aaggggacac tcaatgaagc aattgacatg aaaaccattc ataagttgat atgaaaaatg gttaatattt atggtggtgg tttggagcta ataatttgtg tgttcaagtc tcggtccttc tttttttaac gtttttttt tttcttttat tgggagtgtt 751 801 851 tattgtgtac ttgtaacgta ggccctttgg ttacgcttta agagtttaat 901 aaagăaccac cgttaattta ăăaaaaaaăă aaaaāaaa 951

## F1G.20

#### Chlamydomonas reinhardtii Ipil

(Note: the isomerase cDNA probably ends at ca. base 1103; the second half of the cDNA is similar to extensin and other hydroxyproline-rich structural proteins)

```
ggcacgagct cgagtttgtt ttaccatgac atcgggaatt tggaagcttg
      aactacetca attactcaag taactcgegg caacacattt egegegecat
 51
      cgctgttttc tctgctccag ctaccgagca gcattgcttt agatcgcttt gatgtcataa actcccactt atatgagatc cagtttcatc gagcccaagc
101
151
      ccagagcgca acctgtctta agccgcggca gggcgtccat gcgcctcgcg
201
      caaagccgtg ctctcgttgc gcgtgtcagc tccgccctgt ggccgggagc
251
      aggactttca caggctcaaa gcgttgcggt gcgaatggcg agttcgtcaa
301
      cctgggaagg cacgggcctg agccaggatg acttcatgca gcgggacgag
351
      tgcťťggtgg tggacgacca ggaccggctg ctaggcaccg ccaacaagta
401
      cgactgcac cgcttcgagg cggccaaggg ccagcctgc ggccgcctgc accgcgctt ctccgtgttc ctgttcagcc ccgacggccg actgctgctg
451
501
       cagcagcgcg cagccagcaa ggtgacgttc ccgggtgtgt ggaccaacac
551
       ctgctgctcg cacccgctgg cgggccaggc gccggacgag gtggacctgc
601
      cggcggcggt agcctcgggc caggtgccgg gcatcaaggc ggcggcggtg
cgcaagctgc agcacgagct ggggataccg ccggagcagg ttcccgcctc
651
 701
       ctccttctcc ttcctcacgc gtctgcacta ctgcgccgcc gacaccgcca
751
801
       cgcacggccc ggcggcggag tggggcgagc acgaggtgga ctacgtgctg
       tťcgtgčggc čgcagcagcc cgťcagcctg cagcccaacc cagacgaggť
 851
       ggacgccacg cgctacgtga cgctgccgga gcttcagtcc atgatggcgg
accccggcct cagctggagc ccctggttcc gcatcctggc cacacagccc
 901
 951
       gccttččtgc ccgcctggtg gggcgacctg aagcggcgct ggcgcccggg
1001
       čggcagccga ctgtaggact ggggcaccat ccaccgcgtc atgtgaagaa
1051
       aaaggggaag caggggggg agcgggggat gaatgggaat gtgaatgcga
ttgtgatgcg gcgtgggatg aggtctgaag acagggggaa aatcgggggg
1101
1151
1201
       cgggcgtgag cgtgtgtgta cgtgagcgac aaagccggga ggcggaccgc
       gcgatgggta catgtgtgtg cggagggtcg gtgggtcggt cggttgcgcg gcatagcgtg ttgtgtgtgt gcggctgcgc gggtatgtgg gcacccgggc
1251
1301
       acggaggaga aggcacacgc aggtggcgcg gaggtgtgtc aggggccatg
1351
       ggcgggcctc actcctggtc gtgcccagtg gtctcgtggg cagagtggca
ggggctgcac ccatatgagc ggcgcactgc cgcgctgggc taagtcctta
1401
1451
        tcacttggtg aggtggggcg aggtgggctgt gggcgggggg cgcagtggca gaaggacacg gtgtgtgagc ggtggagctc tggccgtgcc ggccgtgagg ggcggatagc gatatgacgt tgtgcttggc cgctgtaatg cgggagaatg
1501
1551
1601
        tgcaggccgc gagaagcggg cggtggcagg aggccgcagg ctgcagcacc
1651
        cğttğğggag ğtğccğcčtğ caggcgcggc gccgggcggg cctgagtaat
1701
        gggcgcctga gtagtggcgg ccacaggagg cgcaggaggc agcagcagga
ggacgagctg gagggacccg ttggcaaccc aaggttgcgc gtgtaacata
 1751
 1801
        gtggccatac aaaaaaaaaa aaaa
 1851
```

## FIG. 21A

Tagetes erecta Ipil ccaaaaacaa ctcaaatctc ctccgtcgct cttactccgc catgggtgac gactccggca tggatgctgt tcagcgacgt ctcatgtttg acgatgaatg čattttğğtg găfgağtgfg acaătğtgğt gggacătgaf accaaătacă 101 attgtcactt gatggagaag attgaaacag gtaaaatgct gcacagagca 151 201 251 ttcagcgttt ttctattcaa ttcaaaatac gagttacttc ttcagcaacg gtctgcaacc aaggtgacat ttcctttagt atggaccaac acctgttgca gccatccact ctacagagaa tccgagcttg ttcccgaaaa cgcccttgga gtaagaaatg ctgcacagag gaagctgttg gatgaactcg gtatccctgc tgaagatgtt cccgttgatc agtttactcc tttaggtcgc atgctctaca 301 351 401 ağgctccătc tgatggăaag tğgggagaac atgaăcttğa ctăcctactt 451 ttcatagtga gagacgttgc tgtaaacccg aacccagatg aagtggcgga 501 551 tatcaaatat gtganccang aagagttaaa ggagctgcta aggaaagcag atgcggggga ggagggtttg aagctgtctc catggttcag gttagtggtt 601 gaťaččťťčt ťgtťčaagtg gtgggatcat gtgčaaaagg gtacacťčac 651 fgaagcaatt gatatgaaaa ccafacacaa gcfgatatag aaacacaccc tcaaccgaaa agttcaagcc taataattcg ggttgggtcg ggtctaccat 701 751 caattgttt tttctttaa gaagttttaa tctctatttg agcatgttga 801 ttcttgtctt ttgtgtgtaa gattttgggt ttcgtttcag ttgtaataat gaaccattga tggtttgcaa tttcaagttc ctatcgacat gtagtgatct 851 901 951 aaaaaa

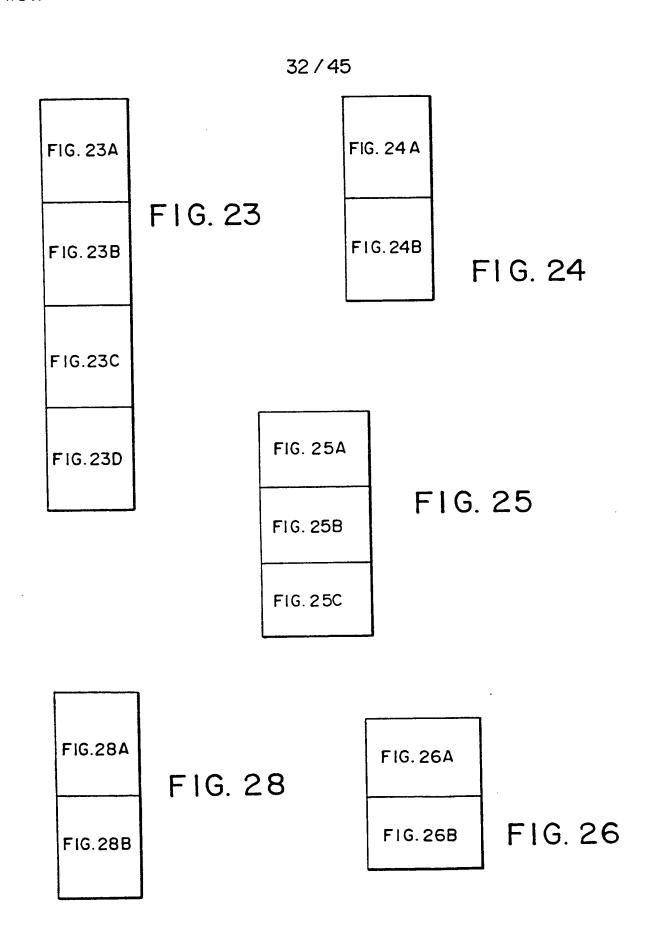
## FIG. 21B

Oryza sative Ipil cctccctttg cctcgcgcag aggcggccgc gccttctccg ccgcgaggat ggccggcgcc gccgccgcg tggaggacgc cgggatggac gaggtccaga agcggctcat gttcgacgac gaatgcattt tggtggatga acaagacaat 101 gttgttggcc atgaatcaaa atataactgc catctgatgg aaaaaatcga 151 201 atctgaaaat ctacttcata gggctttcag tgtattcctg ttcaactcaa aatatgaact cctactccag caacgatctg caacaaaggt tacatttcct 251 301 ctagtttgga ccaacacttg ctgcagccat cctctgtacc gtgagtctga gcttatacag gaaaactacc ttggtgttag aaatgctgct cagaggaagc 351 tcttggatga gctgggcatc ccagctgaag atgtgccagt tgaccaattc 401 acccetettg gtcggatget ttacaaggee ceatetgatg gaaaatgggg tgaacacgag ettgactace tgetgtteat cgtccgcgae gtgaaggtag 451 501 tcccgaaccc ggacgaagtg gccgatgtga aatacgtgag ccgtgagcag ctgaaggagc tcatccgcaa agcggacgcc ggagaggaag gcctgaagct gtctccctgg ttccggctgg ttgttgacaa cttcctcatg ggctggtggg 551 601 651 atcacgtcga gaaaggcacc ctcaacgagg ccgtggacat ggagaccatc cacaagctga agtaaggact gcgatgttgt ggctggaaag aatgatcctg 701 751 aagactctğt tcttgtgctg ctgcatatta ctcttaccag ggaagttgca 801 gaagtcagaa gaagcttttg tatgtttctg ggtttggagc ttggaagtgt tgggctctgc tgactgagag attcccttat agagtgtcta tgttaattta 851 901 gcaaacttct atattataca tgattagtta attgttcggt gtctgaataa 951 agaacaatag catgttccat gtttatttgc t 1001

30/45

	181 195 196	210 211 225 226	226 240	240 241 255	255 256 270	
1 T.erecta 1	AQRKLLDELGIPAED VPVDQFTPLG	VPVDQFTPLGRMLYKAPSDGKWG E	EHELDYLLFIVRD	EHELDYLLFIVRD VAVNPNPDEVADIKY VSHEELKELLRKADA	VSHEELKELLRKADA	88
2 1 cativa 1		KWG	EHELDYLLFMVRD		VGLDPNPDEVKDVKY VNREELKELVRKADA	236
2 / 50411/0 1		KAPSDGKWG	FHFVDYI LFLVRD		VAVNPNPDEVADIRY VNQEELKELLRKADA	88
3 L.SULIVU 2		DISC DO	CHELDYLL CTVDD.		WKYDDNDDEVADAKY VNRFEI KFII RKADA	251
4 A.palaestina 2		DWNDUC	ENECDICLIAND		WINCELL DE TENNO DA	5 6
5 A.palaestina 1	AQRKLLDELGIPAED VPVDEFTPLGRILY-		EHELUYLL' 1VKU		VKYUPNPUEVAUAKY VNKEELKEILKNAUA	<u> </u>
6 O.sativa 1	AQRKLLDELGIPAED VPVDQFTPLGRMLY-	*MLYKAPSDGKWG EHELDYLLFIVRD	EHELDYLLFIVRD		VKVVPNPDEVADVKY VSREQLKELIRKADA	194
7 A.thaliana 1	AQRKLLDELGIVAED VPVDEFTPLGRMLY-	#MLYKAPSDGKWG EHELDYLLFIVRD	EHELDYLLFIVRD		VKVQPNPDEVAEIKY VSREELKELVKKADA	241
8 A.thaliana 2	AQRKLFDELGIVAED VPVDEFTPLGRMLY-	ZMLYKAPSDGKWG EHEVDYLLFIVRD	EHEVDYLLFIVRD		VKLQPNPDEVAEIKY VSREELKELVKKADA	190
9 H.pluvialis 1	AIRKLEHELGIPAHQ LPASAFRFLT	LPASAFRFLTRLHYC AADVQPAATQSALWG EHEMDYILFIRAN	EHEMDYILFIRAN		VTQEELRQMMQP	247
10 H.pluvialis 2	AIRKLEHELGIPAHQ LPASAFRFLT	LPASAFRFLTRLHYC AADVQPAATQSALWG EHEMDYILFIRAN	EHEMDYILFIRAN	<ul> <li>VTLAPNPDEVDEVRY</li> </ul>	VTQEELRQMMQP	529
11 C.reinhardtii 1	AVRKLQHELGIPPEQ VPASSFSFLT	VPASSFSFLTRLHYC AADTATHG-PAAEWG EHEVDYVLFVRPQQP VSLQPNPDEVDATRY VTLPELQSMMA-	EHEVDYVLFVRPQQI	VSLQPNPDEVDATRY	VTLPELQSMMA	259
	785 786	315 315	316			
	3	700 000			(1.1	
1 T.erecta 1		DNFLFKAWDHVQK GTLTEAIDMKII HKLI		lagetes erecta (marigola)	(marigola)	
2 L.sativa 1	GEEGVKLSPWFKLIV DNFLFQWW	DNFLFQWWDRLHK GTLTEAIDMKTI	HKLT 280	Lactuca sativa	Lactuca sativa (romaine lettuce)	
3 L.sativa 2	GEEGLKLSPWFRLVV DNFLFKWW	DNFLFKWWDHVQK GTLNEAIDMKTI	Н 229	Lactuca sativa	Lactuca sativa (romaine lettuce)	
4 A.palaestina 2		DNFLFKWWDHVEE GKIKDVADMKTI	HKLT 295	Adonis palaesti	Adonis palaestina (pheasant's eye)	_
5 A.palaestina 1		DNFLFKWWDHVEQ GTIKEVADMKTI	HKLT 234	Adonis palaesti	Adonis palaestina (pheasant's eye)	_
6 O.sativa 1		DNFLMGWMDHVEK GTLNEAVDMETI	HKLK 238	Oryza sativa (rice,	ice)	
7 A.thaliana 1			HKL 284	Arabidopsis thaliana	liana	
8 A.thaliana 2	GDEAVKLSPWFRLVV DNFLMKWM	DNFLMKWWDHVEK GTITEAADMKTI	HKL 233	Arabidopsis thaliana	liana	
9 H.pluvialis 1	-DNGLQWSPWFRIIA ARFLERWA	ARFLERWADLDA ALNTDKHEDWGTV	HHINEA	Haematococcus pluvialis	luvialis	
10 H.pluvialis 2	-DNGLQWSPWFRIIA ARFLERWA	ARFLERWMADLDA ALNTDKHEDWGTV	HHINEA	Haematococcus pluvialis	luvialis	
11 C. reinhardtii 1	-DPGLSWSPWFRILA TOPAFLPAWM	TOPAFLPAWMGDLKR RWRPGGSRLSDWGTI HRVM	HRVM 307	Chlamydomonas reinhardtii	einhardtii	

# F16.22B



# FIG. 23A

Comparison using GAP program of the Genetics Computer Group Gap Weight: 50 Average match: 10.000 Length Weight: 3 Average Mismatch: 0.000 Quality: 17392 Length: 1904 Ratio: 9.411 Gaps: 3  Percent Similarity: 95.331 Percent Identity: 95.331  Match display thresholds for the alignment(s):    = IDENTITY := 5 . = 1
Adonis palaestina ε-cyclase #3 x Adonis palaestina ε-cyclase #5
1 gagagaaaagagtgttatattaatgttactgtcgcattcttgcaacac. 49
1aaaggagtgttctattaatgttactgtcgcattcttgcaacact 44
50 .atattcagactccattttcttgttttctcttcaaaacaacaactaatg 98
45 tatattcaaactccattttcttcttttctcttcaaaacaac
99 tga.cggagtatctagctatggaactacttggtgttcgcaacctcatctc 147
95 tgagcagagtatctggctatggaactacttggtgttcgcaacctcatctc 144
148 ttcttgccctgtctggacttttggaacaagaaaccttagtagttcaaaac 197
145 ttcttgccctgtgtggacttttggaacaagaaaccttagtagttcaaaac 194
198 tagcttataacatacatcgatatggttcttcttgtagagtagattttcaa 247
195 tagcttataacatacatcgatatggttcttcttgtagagtagattttcaa 244
248 gtgagggctgatggtggaagcgggagtagaacttctgttgcttataaaga 297
245 ģtģāģāģētģātģģtģģāāģēģģģāģtāģāāgttētģttģēttātāāāģā 294
298 gggttttgtggacgaggaggattttatcaaagctggtggttctgagcttt 347
295 ġġġttttġtġġġátġaaġáġġáttttátċáááġċtġġţġţttċtġáġċttt 344
348 tgtttgtccaaatgcagcaaacaaagtctatggagaaacaggccaagctc 397
345 tgtttgtccááátgcágcááácááágtctátggágáááácággccáágctc 394

## FIG. 23B

398	gccgataagttgccaccaatacctttcggagaatctgtgatggacttggt	447
395	gccgataagttgccaccaataccttttggagaatccgtgatggacttggt	444
448	tgtaataggttgtggacctgctggtctttcactggctgcagaagctgcta	497
445	tgtaataggttgtggacctgctggtctttcactggctgcagaagctgcta	494
498	agctaggcttgaaagttggccttattggtcctgatcttccttttacaaat	547
495	agctagggttgaaagttggccttattggtcctgatcttccttttacaaat	544
	aattatggtgtgtgggaagacgagttcaaagatcttggacttgaacgttg	
	aattatggtgtgtgggaagacgagttcaaagatcttggacttgaacgttg	
	tatcgagcatgcttggaaggacaccatcgtatatcttgacaatgatgctc	647
	tatcgagcatgcttggaaggacaccatcgtatatcttgataatgatgctc	644
	ctgtccttattggtcgtgcatatggacgagttagccggcatttgctgcat	697
	ctgtccttattggtcgtgcatatggacgagttagtcgacatttgctacat	694
	<pre>gaagagttgctgaaaaggtgtgtcgagtcaggtgtatcatatctgaattc</pre>	747
	gaggagttgctgaaaaggtgtgtggagtcaggtgtatcatatctggattc	
	taaagtggaaaggatcactgaagctggtgatggccatagtcttgtagttt	797
		794
	gtgaaaacgacatctttatcccttgcaggcttgctactgttgcatctgga	
	<pre>gcagcttcagggaaacttttggagtatgaagtaggtggccctcgtgtttg                       </pre>	897
	• • • • • • • • • • • • • • • • • • • •	894
	tgtccaaactgcttatggtgtggaggttgaggtggagaacaatccatacg	
895	tgtccaaaccgcttatggggtggaggttgaggtggagaacaatccatacg	944

# FIG. 23C

948	atcccaacttaatggtatttatggactacagagactatatgcaacagaaa	997
945	atcccaacttaatggtattcatggactacagagactatatgcaacagaaa	994
998	ttacagtgctcggaagaagaatatccaacatttctctatgtcatgcccat	1047
995	ttacagtgctcggaagaagaatatccaacatttctctatgtcatgcccat	1044
1048	gtcgccaacaagacttttttttgaggaaacctgtttggcctcaaaagatg	1097
1045	gtcgccaacaagacttttttttgaggaaacctgtttggcctcaaaagatg	1094
1098	ccatgcctticgatctactgaagagaaaactaatgtcacgattgaagact	1147
1095	ccatgccattcgatctactgaagagaaaactgatgtcacgattgaagact	1144
1148	ctgggtatccaagttacaaaaatttatgaagaggaatggtcttatattcc	1197
1145	ctgggtatccaagttacaaagtttatgaagaggaatggtcatatattcc	1194
	tgttgggggttctttaccaaacacagagcaaaagaacctagcatttggtg	1247
	tgttggtgttctttaccaaacacagagcaaaagaacctagcatttggtg	1244
	ctgcagcaagcatggtgcatccagcaacaggctattcggttgtacgatca	
	ctgcagcaagcatggtgcatccagcaacaggctattcggttgtacggtca	1294
	ctatcagaagctccaaaatatgcttctgtaattgcaaagattttgaagca	
	ctgtcagaagctccaaaatatgcttctgtaattgcaaagattttgaagca	
	agataactctgcatatgtggtttctggacaaagcagtgcagtaaacattt	
	agataactctgcgtatgtggtttctggacaaagtagtgcagtaaacattt	
	caatgcaagcatggagcagtctttggccaaaggagcgaaaacgtcaaaga	
	caatgcaagcatggagcagtctttggccaaaggagcgaaaacgtcaaaga gcattctttcttttcgggttagagcttattgtgcagctagatattgaagc	
	gcattctttcttttcgggttagagcttattgtgcagctagatattgaagc 	
1440	year corrections and the second secon	

1498	aaccagaacgttctttagaaccttcttccgcttgccaacttggatgtggt	1547
1495	aaccagaacattctttagaaccttcttccgcttgccaacttggatgtggt	1544
1548	ggggtttccttgggtcttcactatcatctttcgatcttgtattgttttcc	1597
1545	ggggtttccttgggtcttcactatcatctttcgatctcgtcttgttttcc	1594
1598	atgtacatgtttgttttggccccgaacagcatgaggatgtcacttgtgag	1647
1595	atgtacatgtttgttttggcgccaaacagcatgaggatgtcacttgtgag	1644
1648	acatttgctttcagatccttctggtgcagttatggttaaagcttacctcg	1697
1645	acatttgctttcagatccttctggtgcagttatggtaagagcttacctcg	1694
1698	aaaggtaatctgttttatgaaactatagtgtctcattaaataaatga	1744
1695	adaggtagtctcatctattattadactctagtgtttcaccadatadatga	1744
1745	ggatccttcgtatatgtatatgatcatctctatgtatatcctatattcta	1794
1745	ggatccttcgaatgtgtatatgatcatctctatgtatatcctgtactcta	1794
	atctcataaagtaatcgaaaattcattgatagaaaaaaaa	
1795	atctcataaagtaaatgccgggtttgatattgttgtgtcaaaccggccaa	
	aaaa	1848
1845	tgatataaagtaaatttattgatacaaaagtagtttttttt	1894

# FIG. 23D

GAP program of Genetics Computer Group FIG. 24A blosum62.cmp
Gap Weight: 12 Average Match: 2.912 Length Weight: 4 Average Mismatch: -2.003 Quality: 2728 Length: 530 Ratio: 5,147 Gaps: 0 Percent Similarity: 99,623 Percent Identity: 99.057 Match display thresholds for the alignment(s):
= IDENTITY := 2 .= 1 Adonis palaestina $\epsilon$ -cyclase #3 x Adonis palaestina $\epsilon$ -cyclase #5
1 MELLGVRNLİSSCPVWTFGTRNLSSSKLAYNIHRYGSSCRVDFQVRADGG 50
51 SGSRTSVAYKEGFVDEEDFİKAGGSELLFVQMQQTKSMEKQAKLADKLPP 100    .
101 IPFGESVMDLVVIGCGPAGLSLAAEAAKLGLKVGLIGPDLPFTNNYGVWE 150
151 DEFKDLGLERCIEHAWKDTİVYLDNDAPVLIGRAYGRVSRHLLHEELLKR 200 
201 CVESGVSYLNSKVERITEAGDGHSLVVCENDIFIPCRLATVASGAASGKL 250
251 LEYEVGGPRVCVQTAYGVEVEVENNPYDPNLMVFMDYRDYMQQKLQCSEE 300
301 EYPTFLYVMPMSPTRLFFEETCLASKDAMPFDLLKRKLMSRLKTLGIQVT 350

351	KIYEEEWSYIPVGGSLPNTEQKNLAFGAAASMVHPATGYSVVRSLSEAPK	400
351	:	400
401	YASVIAKILKODNSAYVVSGQSSAVNISMOAWSSLWPKERKRORAFFLFG	450
401		450
451	LELIVOLDIEATRTFFRTFRLPTWMWWGFLGSSLSSFDLVLFSMYMFVL	500
451		500
	APNSMRMSLVRHLLSDPSGAVMVKAYLER* 530	
501		

FIG. 24B

54 204 204 204 202 202 202 203 191 191 181 182 183 183
KACIAN COLLINATION OF COLUMN OF COLLINATION OF COLUMN OF
XSOWN XSOWN
SSRHL NRKQLELL NRKQLE
200 GRAYGR GRAYGR GRAYGR GRAYGR GRAYGR HRPYGR NRPYGR NRPYGR NRPYGR SRPYGR SRPYGR NRPYGR SRPYG
20 20 21 LIGH 20 LIGH 21 LIGH 21 LIGH 21 LIGH 22 LIGH 23 LIGH 24 LIGH 26 LIGH 26 LIGH 27 LIGH
* 160 * 200
DTIVY DTIVY DTIVY DTIVY SAVYY SAVYY SAVYY
180 ACIEHVAR KCIEHVAR RCIEHVAR
* * * * * * * * * * * * * * * * * * *
WWWDE WWWDE WWW.
160 FTUNY FT
* * * * * * * * * * * * * * * * * * *
KLGLKVGL IGPOLP— KLGLKVGL IGPOLP— KLGLKVGL IGPOLP— KLGLKVGL IGPOLP— KLGLNVGL
140 ESAKL ESAKL ESAKL ESAKL ESAKL ESAKL QVSEA QVSEA QVSEA QVSEA QVSEA QVSEA
SILALAA SILALAA SILSLAA SILSLAA SILALAA SILALAA SILALAA SILALAA SILALAA SILANAA SILANAA SILANAA SILANAA SILANAA
120 * 140  DGALDHWYTGCGPAGLALAAESA ESWIDL WYTGCGPAGLSLAAEAA ESWIDL WYTGCGPAGLSLAAEAA NCTLOL WYTGCGPAGLALAAESA QTVLDL WYTGCGPAGLALAAESA QTVLDL WYTGCGPAGLALAAESA QTVLDL WYTGCGPAGLALAAESA GWYDLAWYGCGPAGLAWAQQWSI SUWDLAWYGCGPAGLAWAQQWSI SUWDLAWYGCGPAGLAWAQQWSI WWYDLWYGCGPAGLAWAQQWSI WWWDLWWGCGPAGLAWAQQWSI WWWDLWWGCGPAGLAWAQQWSI WWWDLWWGCGPAGLAWAQQWSI WWWDLWWGCGPAGLAWAQQWSI WWWDLWWGCGPAGLAWAQQWSI WWWDLWWGCGPAGLAWAQQWSI
LAVVG GAVE
120
120 * 140 * 160 * 160 * 220 *
** ** ** ** ** ** ** ** ** ** ** ** **
FE lopsisl E2 E2 EE E E Opsisk 3 3 3 1dB
PotatoE ArabidopsisE AdonisE1 AdonisE2 LettuceEE TomatoE ArabidopsisB AdonisB PepperB TomatoB TomatoB TomatoB TomatoB DaffodilB



Potatof Signitus (Ginera Programme) 250  Arabidopsis E Signitus (Ginera Programme) 250  Arabidopsis E Signitus (Ginera Programme) 250  Arabidopsis E Signitus (Ginera Programme) 250  Arabidopsis E Signitus (Ginera Programme) 250  Arabidopsis E Signitus (Ginera Programme) 250  Arabidopsis E Signitus (Ginera Programme) 250  Arabidopsis E Signitus (Ginera Programme) 250  Arabidopsis E Signitus (Ginera Programme) 250  Arabidopsis E Signitus (Ginera Programme) 250  Arabidopsis E Signitus (Ginera Programme) 250  Arabidopsis E Signitus (Ginera Programme) 250  Bartool B Signitus (Ginera
1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1
To Para Man A A A L L A A A A L L A A A A L L L A A A A L L L A A A L L L L A A A L

* 240 * 260 * 300 * 300 * 167 YLLSKNORIVEGEDVVIPCREVITVASGAASGKFLOYELGGPRVSVQTAYGVEVEVDNNPFOPSLMVPMDYRDYNPHDAQSLEAKYPTFLYAMPMFRRIF : 167 YLLSKNOSITEASOGLRLVAGODNNVIPCRLATVASGAASGKLLOYEVGGPRVCVQTAYGVEVEVENNPYOPNLMVPMDYRDYNDKRYRSLEAEYPTFLYAMPMFRRIF : 317 YLLDSKVERITEAGDGHSLVVGENETFIPCRLATVASGAASGKLLEYEVGGPRVCVQTAYGVEVEVENNPYOPNLMVPMDYRDYRDYNCXCSEEYPTFLYWMPMSPTRLF : 317 YLLSKNERITEAGDGHSLVVGENETFIPCRLATVASGAASGKLLEYEVGGPRVCVQTAYGVEVEVENNPYOPNLMVPMDYRDYRDYRDYRDYRDYRDYRDYRDYRDYRDYRDYRDYRD	### ### ##############################	* 460 * 480 * 540 STOAWNTLWPOERKRORSFELFGLALITQUDIES IRSTFRAFFRYPKAMOGELGSSISXADLANIFAFWHITAPHOWRRGETRHIESDPTGATLIRTYLTF: 378 SROAWDTLWPPERKRORAFFLFGLALITQUDIESTRAFFRAPFRAPFRAPFRAPFRAPFRAPFRY : 524 SROAWDTLWPPERKRORAFFLFGLELITYQLDIEATRIFFRAPFRAPFRAPFRAPFRAPFRAPFRAPFRAPFRAPFRA
* 280 * 280 SKUDRIUVEATINGHSIUVECEGDUVII PCREVITUASGAASGKELOYELGGPRUSUQTAY SKUDRIUVEATINGHSIUVECEGDUVII PCREVITUASGAASGKELOYELGGPRUSUQTAY SKUER LITEAGDGHSLUVCENE I FII PCREATIVASGAASGKELEYEVGGPRUCYQTAY SKUER LITEAGDGHSLUVCEND I FII PCREATIVASGAASGKELEYEVGGPRUCYQTAY SKUER LITEAGDGHSLUVCEND I FII PCREATIVASGAASGKELOYELGGPRUCYQTAY SKUER LITEAGDGHSLUVECEGDUVII PCREATIVASGAASGKELOYELGGPRUCYQTAY SKUOR I VEATIVASGAASGKELOYELGSPRUSUQTAY	340 * 380 *	* 480 * 500 SITAMATILWPOERKRORSFFLFGLALILQUDES IRSFRAFFRYPKAMOGFLGSSLS SRQANDTILWPPERKRORAFFLFGLALILYQUDES IRSFRAFFRYPKAMOGFLGSSLS SRQANDTILWPPERKRORAFFLFGLELILYQUDES IRSFRAFFRYPKAMOGFLGSSLS SYQANSSLWPKERKRORAFFLFGLELILYQUDES IRSFFRAFFRYPKAMAGFLGSSLS SYQANSTLWPLERKRORAFFLFGLSHIYLWDLEGTRIFFRYFFRYPKAMAGFLGSSLS SYQANFTLWPLERKRORSFFLFGLSHIYLWDLEGTRIFFRYFFRYPKAMAGFLGSSLS SYQANFTLWPLERKRORSFFLFGLSHIYLWDLEGTRIFFRYFFRYPKAMAGFLGSSLS SYQANFTLWPLERKRORSFFLFGLSHIYLWDLEGTRIFFRYFFRYPKAMAGFLGSSLS SYQANFTLWPLERKRORSFFLFGLSHIYLWDLEGTRIFFRYFFRYPKAMAGFLGSSLS
PotatoE : YUN ArabidopsisE : YUN AdonisE1 : YUN AdonisE2 : YUN LettuceEE : YUN TomatoE : YUN	PotatoE : FEI ArabidopsisE : FEI AdonisE1 : FEI LettuceEE : FEI TomatoE : FEI	PotatoE : STA ArabidopsisE : STA AdonisE1 : STA AdonisE2 : STA LettuceEE : STA TomatoE : STA MarigoldE : STA

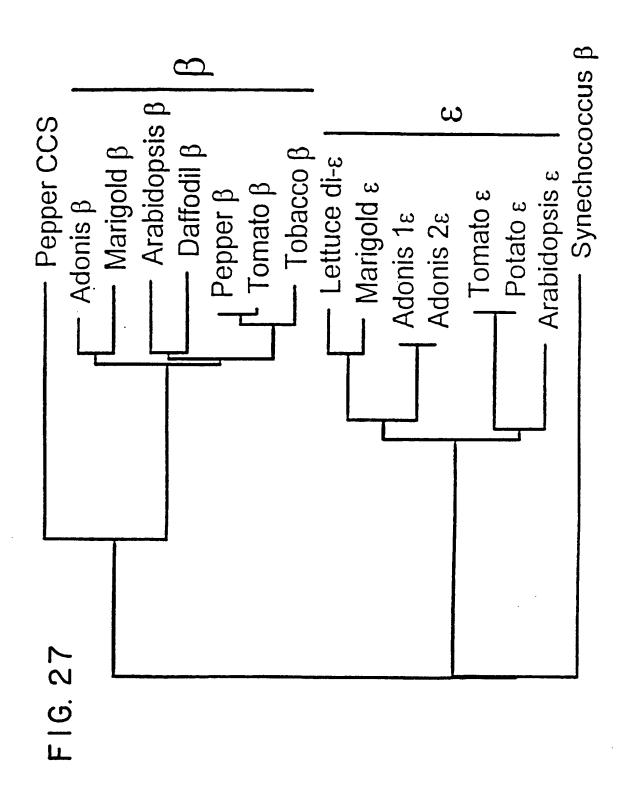
F16.26E





4	11/4	5	
378 5529 553 551 502 503 503 503		102 103 107 109 88	57 208 207 207 212 205 194
STPS-ISTOQQUITUMPGERRROPELFEGAL ILQUDIEG RESFERAFERVRAM GGEGSSISSADI MIFATMETI IAPNUMRRGLIRHLISDFIGATLIRTYLIFNISROQQUITUMPGERRROPELFEGAL ILQUDIEG RESFERAFER PRAMAGGEGSSISSADI MIFATMETI IAPNUMRRGLIRHLISDFIGATMIKTYLKV	*	MECVGARNF-AAMAVSTFPSWS-CRRKFPVVKRYSYRN IRFGL-CSVRASGGGSSGSESCVAVREDFADEEDFYKAGGSETLIFVQWQQNKDWDEQSKLVDAGIPPIS MELLGVRNLISSCPVMT-FGTRNLSSSKLAYN IHRYGSSCRVDFQVRADGGSGSRSSVAYKEGFVDEEDFIKAGGSELLFVQWQQTKSWEKQAKLADKIPPIP MELLGVRNLISSCPVWT-FGTRNLSSSKLAYN IHRYGSSCRVDFQVRADGGSGSRTSVAYKEGFVDEEDFIKAGGSELLFVQWQQTKSWEKQAKLADKIPPIP MECFGARNMTATMAVFTCPRFTDCN IRHKFSLLKQRRFTNLSA-SSSLRQIKCSAKSDRCVVDKQGISVADEEDYVKAGGSELFVQMQTKSWESGSKLSEKLAQIP MECVGVQNV-GAMAVLTRPRLNRWSGGELCQEKSIFLAY-EQYESKCNSSSGSDSCVVDKEDFADEEDYVKAGGSELFVQMQQKGYMDAQSKLSDELRQIS MSMRAG-HMTATMAAFTCPRFMTSIRYTKQIKCNAAKSQLVVKQEIEEEEDYVKAGGSELLFVQMQQNKSYDAQSSLSQKLPRVP	* 160 * 1200 * 220 CONTROLLE CONTROL
RTY! KAY! KAY! KAY! KAY! NNL! NNL!	i	S S S S S S S S S S S S S S S S S S S	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$
TO ATELIA		**************************************	**************************************
2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	100		* # # # # # # # #
		S S S S S S S	**************************************
* IRHI INHI WARHI WARKI WAKHI WAKKI WAKI WA	*	22222	GRV GRV GRV GRV GRV
RECL KKGL KKGL KKGL KKGL KKGL KKGL KKGL KK			200 SRAY( SRAY( SRAY( SRAY(
NDWF NNLF NSWF NSWF NNSWF NNSWF NNSWF NNSWF NNSWF NNSWF NNTSF NNTSF NNTSF NNTSF NNTSF NNTSF NNTSF			
550 11AP VLAP VLAP VLAP 11AP 11AP 11AP 11AP 11AP 11AP 11AP 1	8	XX X X X X X X X X X X X X X X X X X X	ANDAP DOOP DOOP DOOP
YME SLE			7 LD 7 LD 7 LD 7 LD 7 LD 7 LD 7 LD 7 LD
		A V V V V V V V V V V V V V V V V V V V	
	*	265- 265- 265- 261- 261-	WAR A WAR
55 SS SS SS SS SS SS SS SS SS SS SS SS S		AVR VOK	180 CCIEHW CCIEHW CCIEHW CCIEHW CCIEHW
S S S S S S S S S S S S S S S S S S S		ESCV RSSV RTSV RTSV CV	
	9	SGS SGS SGS SGS SGS WKSQ	* 5 5 5 5 5 5 5
PRYY S S S S S S S S S S S S S S S S S S	1	GGGS ADGC CNSS CNSS	
480 LILQLDIEGIRSFFRAFFRLPKWWCLIVQEDIEGIRSFFRAFFFRLPKWWCLIVQLDIEGIRSFFRAFFRLPTWWWCLIVQLDIEGIRSFFRAFFRLPTWWWCLIVQCDIEGIRSFFRAFFRLPTWWWCLILKLDLDATRRFFDAFFDLEPRYWILLKLDLPATRRFFDAFFDLEPRYWILLKLDLPATRRFFDAFFDLEPRYWILLKLDLPATRRFFDAFFDLEPRYWILLKLDLPATRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFDAFFDLEPRYWILLKLDLEGTRRFFAFFAFFAFFAFFAFFAFFAFFAFFAFFAFFAFFAFF	1	-RAS FOVR FOVR FOVR FOVR FOVR	GVE GVE TGVE TGVE
RAFIELD DAFIELD  *	SV- SSLI SSLI	160 NN NN	
		6L-C 6SSC 6SSC 6SSC 8A-S AY-E T	0.09
BO EGT EGT EGT PAT EGT EGT EGT EGT		YRNIRFGL YNIHRYGS YNIHRYGS YNIHRYGS RRFTNLSA EKSIFLAY	31 d d d d d d d d d d d d d d d d d d d
<u> </u>	8	SYRA AYN] AYN] QRRF	N K C C C C C
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\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$		SWS- WIT- NAT- STD	AGI AGI AGI AGI AGI
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		MAVS IS WAVE	
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FIG. 25C  PotatoE : STPS ArabidopsisE : AdonisE1 : SSAV AdonisE1 : SSAV AdonisE1 : SSAV AdonisE1 : SSAV AdonisE1 : SSAV CettuceEE : KYT- FormatoE : SSIP ArabidopsisB : LRGC ArabidopsisB : LRGC AdonisB : -SGN PepperB : -SGN TomatoB : -SGN TomatoB : -SGN MarigoldB : -SGN DaffodilB : -SGN	F 16. 26A		· — — — — — — — — — — — — — — — — — — —
<b>Q</b> Ä Ä	2	isE	isE
GEEE SEI SEI SEI SEI SEI SEI SEI SEI SEI	ہ ج	dops SE1 SE2 CEEE OE	oE dops sE1 sE2 ceEE oE
FG.2 PotatoE ArabidopsisE AdonisE1 LettuceEE TomatoE MarigoldE AdonisB AdonisB PepperB TomatoB TomatoB TomatoB TomatoB TomatoB		ArabidopsisE AdonisE1 AdonisE2 LettuceEE TomatoE	PotatoE ArabidopsisE AdonisE1 AdonisE2 LettuceEE TomatoE MarigoldE
T SAAATEAAATEAAA	- <b>-</b>	· AAAAAA	Z I K A A R

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## FIG. 28A

GAP of: Arabidopsis epsilon cyclase to Lettuce epsilon cyclase Gap Weight: 12
Arabidopsis x Lettuce
1 MECVGARNF.AAMAVSTFPSWSCRRKFPVVKRYSYRNIRFGLCSVRA 46
:  : 1 MECFGARNMTATMAVFTCPRFTDCNIRHKFSLLKQRRFTNLSASSSLRQI 50
47 SGGGSSGSESCVAVREDFADEEDFVKAGGSEILFVQMQQNKDMDEQSKLV 96
:     :     .   :      51 KCSAKSDRCVVDKQGISVADEEDYVKAGGSELFFVQMQRTKSMESQSKLS 100
97 DKLPPISIGDGALDHVVIGCGPAGLALAAESAKLGLKVGLIGPDLPFTNN 146
:     .
147 YGVWEDEFNDLGLQKCIEHVWRETIVYLDDDKPITIGRAYGRVSRRLLHE 196
:     :      :: :
197 ELLRRCVESGVSYLSSKVDSİTEASDGLRLVACDDNNAIPCRLATVASGA 246
201 ELLRRCVESGVSYLSSKVERITEAPNGYSLIECEGNITIPCRLATVASGA 250
247 ASGKLLQYEVGGPRVCVQTAYGVEVEVENSPYDPDQMVFMDYRDYTNEKV 296
:  .       :
297 RSLEAEYPTFLYAMPMTKSRLFFEETCLASKDVMPFDLLKTKLMLRLDTL 346
301 FSI FAKYPTEI YVMAMSPTKI EFFETCI ASDEAMDENI I VSVI MSDI VAM 350

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# FIG. 28B

347	GIRILKTYEEEWSYIPVGGSLPNTEQKNLAFGAAASMVHPATGYSVVRSL	396
351	:	400
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Pro	Asn	Asn	Leu 500	Arg	Lys	Gly	Leu	Ile 505	Asn	His	Leu	Ile	Ser 510	Asp	Pro	
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CCA	CTTAT	CT A	ATCO	GCTGC	CT AT	GTT	STCGF	A GCI	TTGO	TAT	CACT	TCT	ATG (	GCTGT	TATGG	300
CTG'	TTTAC	CTA (	CAGA	гтстс	CT TO	GCA	ATGO	AG0	GGAGG	STGA	GATO	CTCA	ATG :	rtgg <i>i</i>	AATGT	360
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Arg Pro Glu Ser Thr Ser Ser Thr Asn Ala Ile Asp Ala Glu Tyr Leu

Ala Leu Arg Leu Ala Glu Lys Leu Glu Arg Lys Lys Ser Glu Arg Ser

Thr Tyr Leu Ile Ala Ala Met Leu Ser Ser Phe Gly Ile Thr Ser Met

Ala Val Met Ala Val Tyr Tyr Arg Phe Ser Trp Gln Met Glu Gly Gly

Glu Ile Ser Met Leu Glu Met Phe Gly Thr Phe Ala Leu Ser Val Gly 115 120

Ala Ala Val Gly Met Glu Phe Trp Ala Arg Trp Ala His Arg Ala Leu 135 140

Trp His Ala Ser Leu Trp Met Asn His Glu Ser His His Lys Pro Arg

Glu Gly Pro Phe Glu Leu Asn Asp Val Phe Ala Ile Val Asn Ala Gly 170

Pro Ala Ile Gly Leu Leu Ser Tyr Gly Phe Phe Asn Lys Gly Leu Val

Pro Gly Leu Cys Phe Gly Ala Gly Leu Gly Ile Thr Val Phe Gly Ile 200

Ala Tyr Met Phe Val His Asp Gly Leu Val His Lys Arg Phe Pro Val 210 220

Gly Pro Ile Ala Asp Val Pro Tyr Leu Arg Lys Val Ala Ala Ala His 225 230 235 240

Gln Leu His His Thr Asp Lys Phe Asn Gly Val Pro Tyr Gly Leu Phe 245 250 255

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Asn Asp Leu Tyr Gly Val Val Phe Ala Val Leu Ala Thr Ile Leu Phe 50 55 60

Thr Val Gly Ala Tyr Trp Trp Pro Val Leu Trp Trp Ile Ala Leu Gly 65 70 75 80

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His Gln Arg Trp Pro Phe Arg Tyr Ile Pro Arg Arg Gly Tyr Phe Arg 100 105 110

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Pro Ser

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Val Gly Thr Ala Gly Val Trp Pro Leu Gln Trp Ile Gly Cys Gly Met 65 70 75 80

Thr Val Tyr Gly Leu Leu Tyr Phe Leu Val His Asp Gly Leu Val His
85 90 95

Gln Arg Trp Pro Phe His Trp Ile Pro Arg Arg Gly Tyr Leu Lys Arg 100 105 110

Leu Tyr Val Ala His Arg Leu His His Ala Val Arg Gly Arg Glu Gly 115 120 125

Cys Val Ser Phe Gly Phe Ile Tyr Ala Arg Lys Pro Ala Asp Leu Gln 130 135 140

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Gly Trp Gly Trp His Leu Ser His His Glu Pro Arg Lys Gly Ala Phe 35 40 45

Glu Val Asn Asp Leu Tyr Ala Val Val Phe Ala Ala Leu Ser Ile Leu 50 55 60

Leu Ile Tyr Leu Gly Ser Thr Gly Met Trp Pro Leu Gln Trp Ile Gly 65 70 75 80

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Leu Val His Gln Arg Trp Pro Phe Arg Tyr Ile Pro Arg Lys Gly Tyr
100 105 110

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780

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#### PCT/US99/12121 WO 99/63055

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ACGAGCTGGG GATACCAGCG	CACCAGCTGC	CGGCAAGCGC	GTTTCGCTTC	CTCACGCGTT	660
TGCACTACTG TGCCGCGGAC	GTGCAGCCAG	CTGCGACACA	ATCAGCGCTC	TGGGGCGAGC	720
ACGAAATGGA CTACATCTTG	TTCATCCGGG	CCAACGTCAC	CTTGGCGCCC	AACCCTGACG	780
AGGTGGACGA AGTCAGGTAC	GTGACGCAAG	AGGAGCTGCG	GCAGATGATG	CAGCCGGACA	840
ACGGGCTGCA ATGGTCGCCG	TGGTTTCGCA	TCATCGCCGC	GCGCTTCCTT	GAGCGTTGGT	900
GGGCTGACCT GGACGCGGCC	CTAAACACTG	ACAAACACGA	GGATTGGGGA	ACGGTGCATC	960
ACATCAACGA AGCGTGAAAG	CAGAAGCTGC	AGGATGTGAA	GACACGTCAT	GGGGTGGAAT	1020
TGCGTACTTG GCAGCTTCGT	ATCTCCTTTT	TCTGAGACTG.	AACCTGCAGT	CAGGTCCCAC	1080
AAGGTCAGGT AAAATGGCTC	GATAAAATGT	ACCGTCACTT	TTTGTCGCGT	ATACTGAACT	1140
CCAAGAGGTC AAAAAAAAAA	AAAAA				1165

<sup>&</sup>lt;210> 12 <211> 1135 <212> DNA

<sup>&</sup>lt;213> Haematococcus pluvialis

CTCGGTAGCT	GGCCACAATC	GCTATTTGGA	ACCTGGCCCG	GCGGCAGTCC	GATGCCGCGA	60
TGCTTCGTTC	GTTGCTCAGA	GGCCTCACGC	ATATCCCGCG	CGTGAACTCC	GCCCAGCAGC	120
CCAGCTGTGC	ACACGCGCGA	CTCCAGTTTA	AGCTCAGGAG	CATGCAGCTG	CTTTCCGAGG	180
ACCGCACAGA	CCACATGAGG	GGTGCAAGCA	CCTGGGCAGG	CGGGCAGTCG	CAGGATGAGC	240
TGATGCTGAA	GGACGAGTGC	ATCTTGGTAG	ATGTTGAGGA	CAACATCACA	GGCCATGCCA	300
GCAAGCTGGA	GTGTCACAAG	TTCCTACCAC	ATCAGCCTGC	AGGCCTGCTG	CACCGGGCCT	360
TCTCTGTGTT	CCTGTTTGAC	GATCAGGGGC	GACTGCTGCT	GCAACAGCGT	GCACGCTCAA	420
AAATCACCTT	CCCAAGTGTG	TGGACGAACA	CCTGCTGCAG	CCACCCTTTA	CATGGGCAGA	480
CCCCAGATGA	GGTGGACCAA	CTAAGCCAGG	TGGCCGACGG	AACAGTACCT	GGCGCAAAGG	540
CTGCTGCCAT	CCGCAAGTTG	GAGCACGAGC	TGGGGATACC	AGCGCACCAG	CTGCCGGCAA	600
GCGCGTTTCG	CTTCCTCACG	CGTTTGCACT	ACTGTGCCGC	GGACGTGCAG	CCAGCTGCGA	660
CACAATCAGC	GCTCTGGGGC	GAGCACGAAA	TGGACTACAT	CTTGTTCATC	CGGGCCAACG	720
TCACCTTGGC	GCCCAACCCT	GACGAGGTGG	ACGAAGTCAG	GTACGTGACG	CAAGAGGAGC	780
TGCGGCAGAT	GATGCAGCCG	GACAACGGGC	TTCAATGGTC	GCCGTGGTTT	CGCATCATCG	840
CCGCGCGCTT	CCTTGAGCGT	TGGTGGGCTG	ACCTGGACGC	GGCCCTAAAC	ACTGACAAAC	900
ACGAGGATTG	GGGAACGGTG	CATCACATCA	ACGAAGCGTG	AAGGCAGAAG	CTGCAGGATG	960
TGAAGACACG	TCATGGGGTG	GAATTGCGTA	CTTGGCAGCT	TCGTATCTCC	TTTTTCTGAG	1020
ACTGAACCTG	CAGAGCTAGA	GTCAATGGTG	CATCATATTC	ATCGTCTCTC	TTTTGTTTTA	1080
GACTAATCTG	TAGCTAGAGT	CACTGATGAA	TCCTTTACAA	CTTTCAAAAA	AAAAA	1135
<210> 13 <211> 960 <212> DNA <213> Taget	ces erecta					
<400> 13	СТСАААТСТС	CTCCCTCCCT	CBER CBCCCC	CAMCCCHCAC	01.0mggggg	
	TCAGCGACGT					60
						120
	GGGACATGAT					180
	GCACAGAGCA					240
	GTCTGCAACC					300
	CTACAGAGAA					360
	NNNNNNNNN					420
MMMMMMMMM	NNNNNNNNNN	MMMMMMMMM	NNNNNNNNN	MMMMMMMMM	MMMMMMMMMM	400

NNNNNNNNN	NNNNNNNNN	NNNNNNNNN	NNNNNNNNN	NNNNNNNNNN	ииииииииии	540
NNNNNNNNN	ииииииииии	ииииииииии	иииииииии	ииииииииии	иииииииии	600
NNNNNNNNN	NNNNNNNNN	имимимими	иииииииии	ииииииииии	иииииииии	660
NNNNNNNNN	ииииииииии	TCATGTGCAA	AAGGGTACAC	TCACTGAATG	CAATTTGATA	720
TGAAAACCAT	ACACAAGCTG	ATATAGAAAC	ACACCCTCAA	CCGAAAAGCA	AGCCTAATAA	780
TTCGGGTTGG	GTCGGGTCTA	CCATCAATTG	TTTTTTTCTT	TTAACAACTT	TTAATCTCTA	840
TTTGAGCATG	TTGATTCTTG	TCTTTTGTGT	GTAAGATTTT	GGGTTTCGTT	TCAGTTGTAA	900
TAATGAACCA	TTGATGGTTT	GCAATTTCAA	GTTCCTATCG	ACATGTAGTG	АТСТААААА	960

<210> 14

<211> 305

<212> PRT

<213> Haematococcus pluvialis

<400> 14

Met Leu Arg Ser Leu Leu Arg Gly Leu Thr His Ile Pro Arg Val Asn
1 5 10 15

Ser Ala Gln Gln Pro Ser Cys Ala His Ala Arg Leu Gln Phe Lys Leu 20 25 30

Arg Ser Met Gln Met Thr Leu Met Gln Pro Ser Ile Ser Ala Asn Leu 35 40 45

Ser Arg Ala Glu Asp Arg Thr Asp His Met Arg Gly Ala Ser Thr Trp 50 60

Ala Gly Gly Gln Ser Gln Asp Glu Leu Met Leu Lys Asp Glu Cys Ile 65 70 75 80

Leu Val Asp Val Glu Asp Asn Ile Thr Gly His Ala Ser Lys Leu Glu 85 90 95

Cys His Lys Phe Leu Pro His Gln Pro Ala Gly Leu Leu His Arg Ala 100 105 110

Phe Ser Val Phe Leu Phe Asp Asp Gln Gly Arg Leu Leu Gln Gln 115 120 125

Arg Ala Arg Ser Lys Ile Thr Phe Pro Ser Val Trp Thr Asn Thr Cys 130 135 140

Cys Ser His Pro Leu His Gly Gln Thr Pro Asp Glu Val Asp Gln Leu 145 150 155 160

Ser Gln Val Ala Asp Gly Thr Val Pro Gly Ala Lys Ala Ala Ile 165 170 175

Arg Lys Leu Glu His Glu Leu Gly Ile Pro Ala His Gln Leu Pro Ala 180 185 190

Ser Ala Phe Arg Phe Leu Thr Arg Leu His Tyr Cys Ala Ala Asp Val 195 200 205

Gln Pro Ala Ala Thr Gln Ser Ala Leu Trp Gly Glu His Glu Met Asp 215 Tyr Ile Leu Phe Ile Arg Ala Asn Val Thr Leu Ala Pro Asn Pro Asp Glu Val Asp Glu Val Arg Tyr Val Thr Gln Glu Glu Leu Arg Gln Met Met Gln Pro Asp Asn Gly Leu Gln Trp Ser Pro Trp Phe Arg Ile Ile Ala Ala Arg Phe Leu Glu Arg Trp Trp Ala Asp Leu Asp Ala Ala Leu Asn Thr Asp Lys His Glu Asp Trp Gly Thr Val His His Ile Asn Glu Ala 305 <210> 15 <211> 293 <212> PRT <213> Haematococcus pluvialis <400> 15 Met Leu Arg Ser Leu Leu Arg Gly Leu Thr His Ile Pro Arg Val Asn Ser Ala Gln Gln Pro Ser Cys Ala His Ala Arg Leu Gln Phe Lys Leu Arg Ser Met Gln Leu Leu Ser Glu Asp Arg Thr Asp His Met Arg Gly Ala Ser Thr Trp Ala Gly Gly Gln Ser Gln Asp Glu Leu Met Leu Lys Asp Glu Cys Ile Leu Val Asp Val Glu Asp Asn Ile Thr Gly His Ala Ser Lys Leu Glu Cys His Lys Phe Leu Pro His Gln Pro Ala Gly Leu 90 Leu His Arg Ala Phe Ser Val Phe Leu Phe Asp Asp Gln Gly Arg Leu 105 Leu Leu Gln Gln Arg Ala Arg Ser Lys Ile Thr Phe Pro Ser Val Trp Thr Asn Thr Cys Cys Ser His Pro Leu His Gly Gln Thr Pro Asp Glu 135 Val Asp Gln Leu Ser Gln Val Ala Asp Gly Thr Val Pro Gly Ala Lys 155 Ala Ala Ala Ile Arg Lys Leu Glu His Glu Leu Gly Ile Pro Ala His

Gln Leu Pro Ala Ser Ala Phe Arg Phe Leu Thr Arg Leu His Tyr Cys

165

14

180 185 190

Ala Ala Asp Val Gln Pro Ala Ala Thr Gln Ser Ala Leu Trp Gly Glu 195 200 205

His Glu Met Asp Tyr Ile Leu Phe Ile Arg Ala Asn Val Thr Leu Ala 210 215 220

Pro Asn Pro Asp Glu Val Asp Glu Val Arg Tyr Val Thr Gln Glu Glu 225 230 235 240

Leu Arg Gln Met Met Gln Pro Asp Asn Gly Leu Gln Trp Ser Pro Trp 245 250 255

Phe Arg Ile Ile Ala Ala Arg Phe Leu Glu Arg Trp Trp Ala Asp Leu 260 265 270

Asp Ala Ala Leu Asn Thr Asp Lys His Glu Asp Trp Gly Thr Val His 275 280 285

His Ile Asn Glu Ala 290

<210> 16

<211> 284

<212> PRT

<213> Arabidopsis thaliana

<400> 16

Met Ser Val Ser Ser Leu Phe Asn Leu Pro Leu Ile Arg Leu Arg Ser  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Leu Ala Leu Ser Ser Ser Phe Ser Ser Phe Arg Phe Ala His Arg Pro 20 25 30

Leu Ser Ser Ile Ser Pro Arg Lys Leu Pro Asn Phe Arg Ala Phe Ser 35 40 45

Gly Thr Ala Met Thr Asp Thr Lys Asp Ala Gly Met Asp Ala Val Gln 50 55 60

Arg Arg Leu Met Phe Glu Asp Glu Cys Ile Leu Val Asp Glu Thr Asp 65 70 75 80

Arg Val Val Gly His Val Ser Lys Tyr Asn Cys His Leu Met Glu Asn 85 90 95

Ile Glu Ala Lys Asn Leu Leu His Arg Ala Phe Ser Val Phe Leu Phe 100 105 110

Asn Ser Lys Tyr Glu Leu Leu Gln Gln Arg Ser Asn Thr Lys Val 115 120 125

Thr Phe Pro Leu Val Trp Thr Asn Thr Cys Cys Ser His Pro Leu Tyr 130 135 140

Arg Glu Ser Glu Leu Ile Gln Asp Asn Ala Leu Gly Val Arg Asn Ala 145 150 155 160

Ala Gln Arg Lys Leu Leu Asp Glu Leu Gly Ile Val Ala Glu Asp Val 165 170 175

Pro Val Asp Glu Phe Thr Pro Leu Gly Arg Met Leu Tyr Lys Ala Pro 180 185 190

Ser Asp Gly Lys Trp Gly Glu His Glu Leu Asp Tyr Leu Leu Phe Ile 195 200 205

Val Arg Asp Val Lys Val Gln Pro Asn Pro Asp Glu Val Ala Glu Ile 210 215 220

Lys Tyr Val Ser Arg Glu Glu Leu Lys Glu Leu Val Lys Lys Ala Asp 225 230 235 240

Ala Gly Glu Gly Leu Lys Leu Ser Pro Trp Phe Arg Leu Val Val 245 250 255

Asp Asn Phe Leu Met Lys Trp Trp Asp His Val Glu Lys Gly Thr Leu 260 265 270

Val Glu Ala Ile Asp Met Lys Thr Ile His Lys Leu 275 280

<210> 17

<211> 287

<212> PRT

<213> Clarkia breweri

<400> 17

Met Ser Ser Ser Met Leu Asn Phe Thr Ala Ser Arg Ile Val Ser Leu  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Pro Leu Leu Ser Ser Pro Pro Ser Arg Val His Leu Pro Leu Cys Phe 20 25 30

Phe Ser Pro Ile Ser Leu Thr Gln Arg Phe Ser Ala Lys Leu Thr Phe 35 40 45

Ser Ser Gln Ala Thr Thr Met Gly Glu Val Val Asp Ala Gly Met Asp 50 60

Ala Val Gln Arg Arg Leu Met Phe Glu Asp Glu Cys Ile Leu Val Asp 65 70 75 80

Glu Asn Asp Lys Val Val Gly His Glu Ser Lys Tyr Asn Cys His Leu 85 90 95

Met Glu Lys Ile Glu Ser Glu Asn Leu Leu His Arg Ala Phe Ser Val 100 105 110

Phe Leu Phe Asn Ser Lys Tyr Glu Leu Leu Leu Gln Gln Arg Ser Ala 115 120 125

Thr Lys Val Thr Phe Pro Leu Val Trp Thr Asn Thr Cys Cys Ser His 130 135 140

Pro Leu Tyr Arg Glu Ser Glu Leu Ile Asp Glu Asn Cys Leu Gly Val 145 150 155 160

Arg Asn Ala Ala Gln Arg Lys Leu Leu Asp Glu Leu Gly Ile Pro Ala 165 170 175

Glu Asp Leu Pro Val Asp Gln Phe Ile Pro Leu Ser Arg Ile Leu Tyr

180 185 190

Lys Ala Pro Ser Asp Gly Lys Trp Gly Glu His Glu Leu Asp Tyr Leu 195 200 205

Leu Phe Ile Ile Arg Asp Val Asn Leu Asp Pro Asn Pro Asp Glu Val 210 215 220

Ala Glu Val Lys Tyr Met Asn Arg Asp Asp Leu Lys Glu Leu Leu Arg 225 230 235 240

Lys Ala Asp Ala Glu Glu Glu Gly Val Lys Leu Ser Pro Trp Phe Arg 245 250 255

Leu Val Val Asp Asn Phe Leu Phe Lys Trp Trp Asp His Val Glu Lys 260 265 270

Gly Ser Leu Lys Asp Ala Ala Asp Met Lys Thr Ile His Lys Leu 275 280 285

<210> 18

<211> 261

<212> PRT

<213> Arabidopsis thaliana

<400> 18

Thr Gly Pro Pro Pro Arg Phe Pro Ile Arg Ser Pro Val Pro Arg
1 5 10 15

Thr Gln Leu Phe Val Arg Ala Phe Ser Ala Val Thr Met Thr Asp Ser 20 25 30

Asn Asp Ala Gly Met Asp Ala Val Gln Arg Arg Leu Met Phe Glu Asp 35 40 45

Glu Cys Ile Leu Val Asp Glu Asn Asn Arg Val Val Gly His Asp Thr 50 55 60

Lys Tyr Asn Cys His Leu Met Glu Lys Ile Glu Ala Glu Asn Leu Leu 65 70 75 80

His Arg Ala Phe Ser Val Phe Leu Phe Asn Ser Lys Tyr Glu Leu Leu 85 90 95

Leu Gln Gln Arg Ser Lys Thr Lys Val Thr Phe Pro Leu Val Trp Thr
100 105 110

Asn Thr Cys Cys Ser His Pro Leu Tyr Arg Glu Ser Glu Leu Ile Glu 115 120 125

Glu Asn Val Leu Gly Val Arg Asn Ala Ala Gln Arg Lys Leu Phe Asp 130 135

Glu Leu Gly Ile Val Ala Glu Asp Val Pro Val Asp Glu Phe Thr Pro 145 150 155 160

Leu Gly Arg Met Leu Tyr Lys Ala Pro Ser Asp Gly Lys Trp Gly Glu 165 170 175

His Glu Val Asp Tyr Leu Leu Phe Ile Val Arg Asp Val Lys Leu Gln 180 185 190

Pro Asn Pro Asp Glu Val Ala Glu Ile Lys Tyr Val Ser Arg Glu Glu 195 200 205

Leu Lys Glu Leu Val Lys Lys Ala Asp Ala Gly Asp Glu Ala Val Lys 210 220

Leu Ser Pro Trp Phe Arg Leu Val Val Asp Asn Phe Leu Met Lys Trp 225 230 235 240

Trp Asp His Val Glu Lys Gly Thr Ile Thr Glu Ala Ala Asp Met Lys 245 250 255

Thr Ile His Lys Leu 260

<210> 19

<211> 288

<212> PRT

<213> Saccharomyces cerevisiae

<400> 19

Met Thr Ala Asp Asn Asn Ser Met Pro His Gly Ala Val Ser Ser Tyr  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Ala Lys Leu Val Gln Asn Gln Thr Pro Glu Asp Ile Leu Glu Glu Phe 20 25 30

Pro Glu Ile Ile Pro Leu Gln Gln Arg Pro Asn Thr Arg Ser Ser Glu
35 40 45

Thr Ser Asn Asp Glu Ser Gly Glu Thr Cys Phe Ser Gly His Asp Glu 50 55 60

Glu Gln Ile Lys Leu Met Asn Glu Asn Cys Ile Val Leu Asp Trp Asp 65 70 75 80

Asp Asn Ala Ile Gly Ala Gly Thr Lys Lys Val Cys His Leu Met Glu 85 90 95

Asn Ile Glu Lys Gly Leu Leu His Arg Ala Phe Ser Val Phe Ile Phe 100 105 110

Asn Glu Gln Gly Glu Leu Leu Gln Gln Arg Ala Thr Glu Lys Ile 115 120 125

Thr Phe Pro Asp Leu Trp Thr Asn Thr Cys Cys Ser His Pro Leu Cys 130 135 140

Ile Asp Asp Glu Leu Gly Leu Lys Gly Lys Leu Asp Asp Lys Ile Lys 145 150 155 160

Gly Ala Ile Thr Ala Ala Val Arg Lys Leu Asp His Glu Leu Gly Ile 165 170 175

Pro Glu Asp Glu Thr Lys Thr Arg Gly Lys Phe His Phe Leu Asn Arg 180 185 190

Ile His Tyr Met Ala Pro Ser Asn Glu Pro Trp Gly Glu His Glu Ile 195 200 205

Asp Tyr Ile Leu Phe Tyr Lys Ile Asn Ala Lys Glu Asn Leu Thr Val

210 215 220

Asn Pro Asn Val Asn Glu Val Arg Asp Phe Lys Trp Val Ser Pro Asn 225 230 235 240

Asp Leu Lys Thr Met Phe Ala Asp Pro Ser Tyr Lys Phe Thr Pro Trp 245 250 255

Phe Lys Ile Ile Cys Glu Asn Tyr Leu Phe Asn Trp Trp Glu Gln Leu 260 265 270

Asp Asp Leu Ser Glu Val Glu Asn Asp Arg Gln Ile His Arg Met Leu 275 280 285

<210> 20

<211> 456

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Consensus
 sequence of four plant B-cyclases

<400> 20

Met Asp Thr Leu Leu Lys Thr Pro Asn Leu Glu Phe Leu Pro His Gly
1 5 10 15

Phe Val Lys Ser Phe Ser Lys Phe Gly Lys Cys Glu Gly Val Cys Val
20 25 30

Lys Ser Ser Ala Leu Leu Glu Leu Val Pro Glu Thr Lys Lys Glu Asn  $35 \hspace{1cm} 40 \hspace{1cm} 45$ 

Leu Asp Phe Glu Leu Pro Met Tyr Asp Pro Ser Lys Gly Val Val Asp 50 55 60

Leu Ala Val Val Gly Gly Pro Ala Gly Leu Ala Val Ala Gln Gln 65 70 75 80

Val Ser Glu Ala Gly Leu Ser Val Cys Ser Ile Asp Pro Pro Lys Leu 85 90 95

Ile Trp Pro Asn Asn Tyr Gly Val Trp Val Asp Glu Phe Glu Ala Met 100 105 110

Asp Leu Leu Asp Cys Leu Asp Ala Thr Trp Ser Gly Ala Val Tyr Ile 115 120 125

Asp Asp Thr Lys Asp Leu Arg Pro Tyr Gly Arg Val Asn Arg Lys Gln 130 135

Leu Lys Ser Lys Met Met Gln Lys Cys Ile Asn Gly Val Lys Phe His 145 150 155 160

Gln Ala Lys Val Ile Lys Val Ile His Glu Glu Lys Ser Met Leu Ile 165 170 175

Cys Asn Asp Gly Thr Ile Gln Ala Thr Val Val Leu Asp Ala Thr Gly 180 185

Phe Ser Arg Leu Val Gln Tyr Asp Lys Pro Tyr Asn Pro Gly Tyr Gln

195 200 205

Val Ala Tyr Gly Ile Leu Ala Glu Val Glu Glu His Pro Phe Asp Lys 210 215 220

Met Val Phe Met Asp Trp Arg Asp Ser His Leu Asn Asn Glu Leu Lys 225 230 235 240

Glu Arg Asn Ser Ile Pro Thr Phe Leu Tyr Ala Met Pro Phe Ser Ser 245 250 255

Asn Arg Ile Phe Leu Glu Glu Thr Ser Leu Val Ala Arg Pro Gly Leu 260 265 270

Arg Met Asp Asp Ile Gln Glu Arg Met Val Ala Arg Leu His Leu Gly 275 280 285

Ile Lys Val Lys Ser Ile Glu Glu Asp Glu His Cys Val Ile Pro Met 290 295 300

Gly Gly Pro Leu Pro Val Leu Pro Gln Arg Val Val Gly Ile Gly Gly 305 310 315 320

Thr Ala Gly Met Val His Pro Ser Thr Gly Tyr Met Val Ala Arg Thr 325 330 335

Leu Ala Ala Pro Val Val Ala Asn Ala Ile Ile Tyr Leu Gly Ser 340 345 350

Glu Ser Ser Gly Glu Leu Ser Ala Glu Val Trp Lys Asp Leu Trp Pro 355 360 365

Ile Glu Arg Arg Arg Gln Arg Glu Phe Phe Cys Phe Gly Met Asp Ile 370 375 380

Leu Leu Lys Leu Asp Leu Pro Ala Thr Arg Arg Phe Phe Asp Ala Phe 385 390 395 400

Phe Asp Leu Glu Pro Arg Tyr Trp His Gly Phe Leu Ser Ser Arg Leu 405 410 415

Phe Leu Pro Glu Leu Ile Val Phe Gly Leu Ser Leu Phe Ser His Ala 420 425 430

Ser Asn Thr Ser Arg Glu Ile Met Thr Lys Gly Thr Pro Leu Val Met 435 440 445

Ile Asn Asn Leu Leu Gln Asp Glu 450 455

<210> 21

<211> 524

<212> PRT

<213> Arabidopsis thaliana

<400> 21

Met Glu Cys Val Gly Ala Arg Asn Phe Ala Ala Met Ala Val Ser Thr 1 5 10 15

Phe Pro Ser Trp Ser Cys Arg Arg Lys Phe Pro Val Val Lys Arg Tyr 20 25 30

Ser Tyr Arg Asn Ile Arg Phe Gly Leu Cys Ser Val Arg Ala Ser Gly Gly Gly Ser Ser Gly Ser Glu Ser Cys Val Ala Val Arg Glu Asp Phe Ala Asp Glu Glu Asp Phe Val Lys Ala Gly Gly Ser Glu Ile Leu Phe Val Gln Met Gln Gln Asn Lys Asp Met Asp Glu Gln Ser Lys Leu Val Asp Lys Leu Pro Pro Ile Ser Ile Gly Asp Gly Ala Leu Asp His Val Val Ile Gly Cys Gly Pro Ala Gly Leu Ala Leu Ala Ala Glu Ser Ala Lys Leu Gly Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Glu Asp Glu Phe Asn Asp Leu Gly Leu Gln Lys Cys Ile Glu His Val Trp Arg Glu Thr Ile Val Tyr Leu Asp Asp Asp Lys Pro Ile Thr Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg Arg 185 Leu Leu His Glu Glu Leu Leu Arg Arg Cys Val Glu Ser Gly Val Ser Tyr Leu Ser Ser Lys Val Asp Ser Ile Thr Glu Ala Ser Asp Gly Leu Arg Leu Val Ala Cys Asp Asp Asn Asn Val Ile Pro Cys Arg Leu Ala Thr Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Gln Tyr Glu Val Gly Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu Asn Ser Pro Tyr Asp Pro Asp Gln Met Val Phe Met Asp Tyr Arg Asp Tyr Thr Asn Glu Lys Val Arg Ser Leu Glu Ala Glu Tyr Pro Thr Phe Leu Tyr Ala Met Pro Met Thr Lys Ser Arg Leu Phe Phe Glu 305 Glu Thr Cys Leu Ala Ser Lys Asp Val Met Pro Phe Asp Leu Leu Lys 330 Thr Lys Leu Met Leu Arg Leu Asp Thr Leu Gly Ile Arg Ile Leu Lys Thr Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro

		355					360					365				
Asn	Thr 370	Glu	Gln	Lys	Asn	Leu 375	Ala	Phe	Gly	Ala	Ala 380	Ala	Ser	Met	Val	
His 385	Pro	Ala	Thr	Gly	Tyr 390	Ser	Val	Val	Arg	Ser 395	Leu	Ser	Glu	Ala	Pro 400	
Lys	Tyr	Ala	Ser	Val 405	Ile	Ala	Glu	Ile	Leu 410	Arg	Glu	Glu	Thr	Thr 415	Lys	
Gln	Ile	Asn	Ser 420	Asn	Ile	Ser	Arg	Gln 425	Ala	Trp	Asp	Thr	Leu 430	Trp	Pro	
Pro	Glu	Arg 435		Arg	Gln	Arg	Ala 440	Phe	Phe	Leu	Phe	Gly 445	Leu	Ala	Leu	
Ile	Val 450	Gln	Phe	Asp	Thr	Glu 455	Gly	Ile	Arg	Ser	Phe 460	Phe	Arg	Thr	Phe	
Phe 465	Arg	Leu	Pro	Lys	Trp 470	Met	Trp	Gln	Gly	Phe 475	Leu	Gly	Ser	Thr	Leu 480	
Thr	Ser	Gly	Asp	Leu 485	Val	Leu	Phe	Ala	Leu 490	Tyr	Met	Phe	Val	Ile 495	Ser	
Pro	Asn	Asn	Leu 500		Lys	Gly	Leu	Ile 505	Asn	His	Leu	ılle	Ser 510	Asp	Pro	
Thr	Gly	Ala 515		Met	Ile	. Lys	Thr 520	Туг	: Leu	Lys	Val	-				
<21 <21	0> 2 .1> 1 .2> I	.898 NA	is pa	alaes	stina	a										
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															IGGAACT	120
															GAAACCT	180
															TAGATTT	240
															AGGGTTT	300
															AAATGCA	360
															TACCTTT	
															CACTGGC	
															CTTTTAC	
															'GTATCGA	
															TTGGTCG	
G	MIG/	11100														

720

TGCATATGGA CGAGTTAGTC GACATTTGCT ACATGAGGAG TTGCTGAAAA GGTGTGTGGA

WA 00/63055	PCT/US99/12121
XX/A 00/63055	• • • • • • • • • • • • • • • • • • • •

GTCAGGTGTA	TCATATCTTG	ATTCTAAAGT	GGAAAGGATC	ACTGAAGCTG	GTGATGGCCA	780
TAGCCTTGTA	GTTTGTGAAA	ATGAGATCTT	TATCCCTTGC	AGGCTTGCTA	CTGTTGCATC	840
TGGAGCAGCT	TCAGGGAAAC	TTTTGGAGTA	TGAAGTAGGT	GGCCCTCGTG	TTTGTGTCCA	900
AACCGCTTAT	GGGGTGGAGG	TTGAGGTGGA	GAACAATCCA	TACGATCCCA	ACTTAATGGT	960
ATTCATGGAC	TACAGAGACT	ATATGCAACA	GAAATTACAG	TGCTCGGAAG	AAGAATATCC	1020
AACATTTCTC	TATGTCATGC	CCATGTCGCC	AACAAGACTT	TTTTTTGAGG	AAACCTGTTT	1080
GGCCTCAAAA	GATGCCATGC	CATTCGATCT	ACTGAAGAGA	AAACTGATGT	CACGATTGAA	1140
GACTCTGGGT	ATCCAAGTTA	CAAAAGTTTA	TGAAGAGGAA	TGGTCATATA	TTCCTGTTGG	1200
TGGTTCTTTA	CCAAACACAG	AGCAAAAGAA	CCTAGCATTT	GGTGCTGCAG	CAAGCATGGT	1260
GCATCCAGCA	ACAGGCTATT	CGGTTGTACG	GTCACTGTCA	GAAGCTCCAA	AATATGCTTC	1320
TGTAATTGCA	AAGATTTTGA	AGCAAGATAA	CTCTGCGTAT	GTGGTTTCTG	GACAAAGTAG	1380
TGCAGTAAAC	ATTTCAATGC	AAGCATGGAG	CAGTCTTTGG	CCAAAGGAGC	GAAAACGTCA	1440
AAGAGCATTC	TTTCTTTTTG	GATTAGAGCT	TATTGTGCAG	CTAGATATTG	AAGCAACCAG	1500
AACATTCTTT	AGAACCTTCT	TCCGCTTGCC	AACTTGGATG	TGGTGGGGTT	TCCTTGGGTC	1560
TTCACTATCA	TCTTTCGATC	TCGTCTTGTT	TTCCATGTAC	ATGTTTGTTT	TGGCGCCAAA	1620
CAGCATGAGG	ATGTCACTTG	TGAGACATTT	GCTTTCAGAT	CCTTCTGGTG	CAGTTATGGT	1680
AAGAGCTTAC	CTCGAAAGGT	AGTCTCATCT	ATTATTAAAC	TCTAGTGTTT	CACCAAATAA	1740
ATGAGGATCC	TTCGAATGTG	TATATGATCA	TCTCTATGTA	TATCCTGTAC	TCTAATCTCA	1800
TAAAGTAAAT	GCCGGGTTTG	ATATTGTTGT	GTCAAACCGG	CCAATGATAT	AAAGTAAATT	1860
TATTGATACA	AAAGTAGTTT	TTTTCCTTAA	AAAAAAA			1898

<sup>&</sup>lt;210> 23

<400> 23

<sup>&</sup>lt;211> 529

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Adonis palaestina

Met Glu Leu Leu Gly Val Arg Asn Leu Ile Ser Ser Cys Pro Val Trp
1 5 10 15

Thr Phe Gly Thr Arg Asn Leu Ser Ser Ser Lys Leu Ala Tyr Asn Ile 20 25 30

His Arg Tyr Gly Ser Ser Cys Arg Val Asp Phe Gln Val Arg Ala Asp 35 40 45

Gly Gly Ser Gly Ser Arg Ser Ser Val Ala Tyr Lys Glu Gly Phe Val 50 55 60

Asp Glu Glu Asp Phe Ile Lys Ala Gly Gly Ser Glu Leu Leu Phe Val 65 70 75 80

Gln Met Gln Gln Thr Lys Ser Met Glu Lys Gln Ala Lys Leu Ala Asp Lys Leu Pro Pro Ile Pro Phe Gly Glu Ser Val Met Asp Leu Val Val Ile Gly Cys Gly Pro Ala Gly Leu Ser Leu Ala Ala Glu Ala Ala Lys Leu Gly Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Glu Arg Cys Ile Glu His Ala Trp Lys Asp Thr Ile Val Tyr Leu Asp Asn Asp 170 Ala Pro Val Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu Leu His Glu Glu Leu Leu Lys Arg Cys Val Glu Ser Gly Val Ser Tyr Leu Asp Ser Lys Val Glu Arg Ile Thr Glu Ala Gly Asp Gly His Ser Leu Val Val Cys Glu Asn Glu Ile Phe Ile Pro Cys Arg Leu Ala Thr 225 235 Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Glu Tyr Glu Val Gly Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu Asn Asn Pro Tyr Asp Pro Asn Leu Met Val Phe Met Asp Tyr Arg Asp Tyr Met Gln Gln Lys Leu Gln Cys Ser Glu Glu Glu Tyr Pro Thr Phe Leu Tyr Val Met Pro Met Ser Pro Thr Arg Leu Phe Phe Glu Glu 310 315 Thr Cys Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Arg 325 Lys Leu Met Ser Arg Leu Lys Thr Leu Gly Ile Gln Val Thr Lys Val 345 Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn 355 Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys Tyr Ala Ser Val Ile Ala Lys Ile Leu Lys Gln Asp Asn Ser Ala Tyr

				405					410					415		
Val	Val	Ser	Gly 420	Gln	Ser	Ser	Ala	Val 425	Asn	Ile	Ser	Met	Gln 430	Ala	Trp	
Ser	Ser	Leu 435	Trp	Pro	Lys	Glu	Arg 440	Lys	Arg	Gln	Arg	Ala 445	Phe	Phe	Leu	
Phe	Gly 450	Leu	Glu	Leu	Ile	Val 455	Gln	Leu	Asp	Ile	Glu 460	Ala	Thr	Arg	Thr	
Phe 465	Phe	Arg	Thr	Phe	Phe 470	Arg	Leu	Pro	Thr	Trp 475	Met	Trp	Trp	Gly	Phe 480	
Leu	Gly	Ser	Ser	Leu 485	Ser	Ser	Phe	Asp	Leu 490	Val	Leu	Phe	Ser	Met 495	Tyr	
Met	Phe	Val	Leu 500	Ala	Pro	Asn	Ser	Met 505	Arg	Met	Ser	Leu	Val 510	Arg	His	
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GATA	CCAT	TG T	ratai	CTT	SA TO	SATGA	ATGAT	יסט יו	TATTO	CTTA	TTG	GCCGT	rgc	CTATO	GAAGA	A 120
GTTA	GTC	SCC A	ATTT <i>P</i>	ACTGO	CA CO	SAGG	AGTT <i>I</i>	A CTO	CAAA	AGGT	GTGT	rggac	GGC .	AGGTO	STTTTC	S 180
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GGTT	TATTO	CAG :	CGTO	CAGAT	C AC	CTGT	CTGAZ	A GCI	rcca <i>i</i>	TAA	GCG	ССТТО	CGT	GCTT	CAAAT	780
ATA	TACO	SAC A	'AAAA	CATA	AG CA	\AGA/	TATO	G CTT	ract?	AGTT	CAAC	STAC	ccc	GAGTA	ATTTC <i>I</i>	A 840

900

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WO 99/63055 FCT/6	0399/121
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- Ser Gly Lys Phe Leu Gln Tyr Glu Leu Gly Gly Pro Arg Val Ser Val 100 105 110
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115 120 125

Ala Glu Ser Ala Lys Leu Gly Leu Asn Val Gly Leu Ile Gly Pro Asp 135 Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Gln Asp Glu Phe Ile Gly 155 Leu Gly Leu Glu Gly Cys Ile Glu His Ser Trp Lys Asp Thr Leu Val Tyr Leu Asp Asp Ala Asp Pro Ile Arg Ile Gly Arg Ala Tyr Gly Arg Val His Arg Asp Leu Leu His Glu Glu Leu Leu Arg Arg Cys Val Glu Ser Gly Val Ser Tyr Leu Ser Ser Lys Val Glu Arg Ile Thr Glu Ala Pro Asn Gly Tyr Ser Leu Ile Glu Cys Glu Gly Asn Ile Thr Ile Pro Cys Arg Leu Ala Thr Val Ala Ser Gly Ala Ala Ser Gly Lys Phe Leu Glu Tyr Glu Leu Gly Gly Pro Arg Val Ser Val Gln Thr Ala Tyr Gly 265 Val Glu Val Glu Val Asp Asn Asn Pro Phe Asp Pro Ser Leu Met Val Phe Met Asp Tyr Arg Asp Tyr Val Arg His Asp Ala Gln Ser Leu Glu Ala Lys Tyr Pro Thr Phe Leu Tyr Ala Met Pro Met Ser Pro Thr Arg 315 Val Phe Phe Glu Glu Thr Cys Leu Ala Ser Lys Asp Ala Met Pro Phe 330 Asp Leu Leu Lys Lys Leu Met Leu Arg Leu Asn Thr Leu Gly Val Arg Ile Lys Glu Ile Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly 360 Gly Ser Leu Pro Asn Thr Glu Gln Lys Thr Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys Cys Ala Phe Val Leu Ala Asn Ile Leu Arg Gln Asn His Ser Lys Asn Met Leu Thr Ser Ser Ser Thr Pro Ser Ile Ser 425 Thr Gln Ala Trp Asn Thr Leu Trp Pro Gln Glu Arg Lys Arg Gln Arg 440

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Gly Ile Arg Ser Phe Phe Arg Ala Phe Phe Arg Val Pro Lys Trp Met 465 470 475 480

Trp Gln Gly Phe Leu Gly Ser Ser Leu Ser Xaa Ala Asp Leu Met Leu 485 490 495

Phe Ala Phe Tyr Met Phe Ile Ile Ala Pro Asn Asp Met Arg Arg Gly 500 505 510

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Gly Arg Ala Tyr Gly Arg Val Ser Arg Arg Leu Leu His Glu Glu Leu 35 40 45

Leu Arg Arg Cys Val Glu Ser Gly Val Ser Tyr Leu Ser Ser Lys Val 50 55 60

Asp Ser Ile Thr Glu Ala Ser Asp Gly Leu Arg Leu Val Ala Cys Asp 65 70 75 80

Asp Asn Asn Val Ile Pro Cys Arg Leu Ala Thr Val Ala Ser Gly Ala 85 90 95

Ala Ser Gly Lys Leu Leu Gln Tyr Glu Val Gly Gly Pro Arg Val Cys 100 105 110

Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu Asn Ser Pro Tyr 115 120 125

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Lys Val Arg Ser Leu Glu Ala Glu Tyr Pro Thr Phe Leu Tyr Ala Met 145 150 155 160

Pro Met Thr Lys Ser Arg Leu Phe Phe Glu Glu Thr Cys Leu Ala Ser 165 170 175

Lys Asp Val Met Pro Phe Asp Leu Leu Lys Thr Lys Leu Met Leu Arg 180 185 190

Leu Asp Thr Leu Gly Ile Arg Ile Leu Lys Thr Tyr Glu Glu Glu Trp

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						120
•	TGGTGGATGA					180
CAICIGAIGG	AAAAAATCGA	ATCIGAAAAT	CIACITCATA	GGGCTTTCAG	TGTATTCCTG	240

TTCAACTCAA AATATGAACT CCTACTCCAG CAACGATCTG CAACAAAGGT TACATTTCCT	300
CTAGTTTGGA CCAACACTTG CTGCAGCCAT CCTCTGTACC GTGAGTCTGA GCTTATACAG	360
GAAAACTACC TTGGTGTTAG AAATGCTGCT CAGAGGAAGC TCTTGGATGA GCTGGGCATC	420
CCAGCTGAAG ATGTGCCAGT TGACCAATTC ACCCCTCTTG GTCGGATGCT TTACAAGGCC	480
CCATCTGATG GAAAATGGGG TGAACACGAG CTTGACTACC TGCTGTTCAT CGTCCGCGAC	540
GTGAAGGTAG TCCCGAACCC GGACGAAGTG GCCGATGTGA AATACGTGAG CCGTGAGCAG	600
CTGAAGGAGC TCATCCGCAA AGCGGACGCC GGAGAGGAAG GCCTGAAGCT GTCTCCCTGG	660
TTCCGGCTGG TTGTTGACAA CTTCCTCATG GGCTGGTGGG ATCACGTCGA GAAAGGCACC	720
CTCAACGAGG CCGTGGACAT GGAGACCATC CACAAGCTGA AGTAAGGACT GCGATGTTGT	780
GGCTGGAAAG AATGATCCTG AAGACTCTGT TCTTGTGCTG CTGCATATTA CTCTTACCAG	840
GGAAGTTGCA GAAGTCAGAA GAAGCTTTTG TATGTTTCTG GGTTTGGAGC TTGGAAGTGT	900
TGGGCTCTGC TGACTGAGAG ATTCCCTTAT AGAGTGTCTA TGTTAATTTA GCAAACTTCT	960
ATATTATACA TGATTAGTTA ATTGTTCGGT GTCTGAATAA AGAACAATAG CATGTTCCAT	1020
GTTTATTTGC T	1031

<210> 36

<211> 232

<212> PRT

<213> Tagetes erecta

<400> 36

Met Gly Asp Asp Ser Gly Met Asp Ala Val Gln Arg Arg Leu Met Phe  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Asp Asp Glu Cys Ile Leu Val Asp Glu Cys Asp Asn Val Val Gly His 20 25 30

Asp Thr Lys Tyr Asn Cys His Leu Met Glu Lys Ile Glu Thr Gly Lys 35 40 45

Met Leu His Arg Ala Phe Ser Val Phe Leu Phe Asn Ser Lys Tyr Glu 50 55 60

Leu Leu Gln Gln Arg Ser Ala Thr Lys Val Thr Phe Pro Leu Val 65 70 75 80

Trp Thr Asn Thr Cys Cys Ser His Pro Leu Tyr Arg Glu Ser Glu Leu 85 90 95

Val Pro Glu Asn Ala Leu Gly Val Arg Asn Ala Ala Gln Arg Lys Leu 100 105 110

Leu Asp Glu Leu Gly Ile Pro Ala Glu Asp Val Pro Val Asp Gln Phe 115 120 125

Thr Pro Leu Gly Arg Met Leu Tyr Lys Ala Pro Ser Asp Gly Lys Trp 130 135 140

Gly Glu His Glu Leu Asp Tyr Leu Leu Phe Ile Val Arg Asp Val Ala 150 Val Asn Pro Asn Pro Asp Glu Val Ala Asp Ile Lys Tyr Val Ser His Glu Glu Leu Lys Glu Leu Leu Arg Lys Ala Asp Ala Gly Glu Gly Leu Lys Leu Ser Pro Trp Phe Arg Leu Val Val Asp Asn Phe Leu Phe Lys Trp Trp Asp His Val Gln Lys Gly Thr Leu Thr Glu Ala Ile Asp 215 Met Lys Thr Ile His Lys Leu Ile <210> 37 <211> 280 <212> PRT <213> Lactuca Sativa <400> 37 Met Leu Lys Phe Pro Pro Phe Lys Thr Ile Ala Thr Met Ile Ser Ser Pro Tyr Ser Ser Phe Leu Leu Pro Arg Lys Ser Ser Phe Pro Pro Met Pro Ser Leu Ala Ala Ala Ser Val Phe Leu His Pro Leu Ser Ser Ala Ala Met Gly Asp Ser Ser Met Asp Ala Val Gln Arg Arg Leu Met Phe Asp Asp Glu Cys Ile Leu Val Asp Glu Asn Asp Lys Val Val Gly His Asp Thr Lys Tyr Asn Cys His Leu Met Glu Lys Ile Glu Lys Gly Asn Met Leu His Arg Ala Phe Ser Val Phe Leu Phe Asn Ser Lys Tyr Glu 105 Leu Leu Leu Gln Gln Arg Ser Ala Thr Lys Val Thr Phe Pro Leu Val Trp Thr Asn Thr Cys Cys Ser His Pro Leu Tyr Arg Glu Ser Glu Leu Ile Asp Glu Asn Ala Leu Gly Val Arg Asn Ala Ala Gln Arg Lys Leu

Gly Glu His Glu Leu Asp Tyr Leu Leu Phe Met Val Arg Asp Val Gly

Leu Asp Glu Leu Gly Ile Pro Gly Ala Asp Val Pro Val Asp Glu Phe

Thr Pro Leu Gly Arg Ile Leu Tyr Lys Ala Ala Ser Asp Gly Lys Trp

195 200 205

Leu Asp Pro Asn Pro Asp Glu Val Lys Asp Val Lys Tyr Val Asn Arg 210 215 220

Glu Glu Leu Lys Glu Leu Val Arg Lys Ala Asp Ala Gly Glu Glu Gly 225 230 235

Val Lys Leu Ser Pro Trp Phe Lys Leu Ile Val Asp Asn Phe Leu Phe 245 250 255

Gln Trp Trp Asp Arg Leu His Lys Gly Thr Leu Thr Glu Ala Ile Asp 260 265 270

Met Lys Thr Ile His Lys Leu Thr 275 280

<210> 38

<211> 229

<212> PRT

<213> Lactuca Sativa

<400> 38

Met Gly Asp Asp Ser Gly Met Asp Ala Val Gln Arg Arg Leu Met Phe 1 5 10 15

Asp Asp Glu Cys Ile Leu Val Asp Glu Asn Asp Asn Val Leu Gly His
20 25 30

Asp Thr Lys Tyr Asn Cys His Leu Met Glu Lys Ile Glu Lys Asp Asn 35 40 45

Leu Leu His Arg Ala Phe Ser Val Phe Leu Phe Asn Ser Lys Tyr Glu 50 55 60

Leu Leu Gln Gln Arg Ser Glu Thr Lys Val Thr Phe Pro Leu Val 65 70 75 80

Trp Thr Asn Thr Cys Cys Ser His Pro Leu Tyr Arg Glu Ser Glu Leu 85 90 95

Ile Pro Glu Asn Ala Leu Gly Val Arg Asn Ala Ala Gln Arg Lys Leu 100 105 110

Leu Asp Glu Leu Gly Ile Pro Ala Glu Asp Val Pro Val Asp Glu Phe 115 120 125

Thr Thr Leu Gly Arg Met Leu Tyr Lys Ala Pro Ser Asp Gly Lys Trp 130 135 140

Gly Glu His Glu Val Asp Tyr Leu Leu Phe Leu Val Arg Asp Val Ala 145 150 155 160

Val Asn Pro Asn Pro Asp Glu Val Ala Asp Ile Arg Tyr Val Asn Gln 165 170 175

Glu Glu Leu Lys Glu Leu Leu Arg Lys Ala Asp Ala Gly Glu Gly 180 185 190

Leu Lys Leu Ser Pro Trp Phe Arg Leu Val Val Asp Asn Phe Leu Phe 195 200 205

Lys Trp Trp Asp His Val Gln Lys Gly Thr Leu Asn Glu Ala Ile Asp 210 215 220

Met Lys Thr Ile His 225

<210> 39

<211> 295

<212> PRT

<213> Adonis Palaestina

<400> 39

Met Ser Ser Ile Arg Ile Asn Pro Leu Tyr Ser Ile Phe Ser Thr Thr  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Thr Lys Thr Leu Ser Ala Ser Cys Ser Ser Pro Ala Val His Leu Gln
20 25 30

Gln Arg Cys Arg Thr Leu Ser Ile Ser Ser Ser Ile Thr Asn Ser Pro 35 40 45

Arg Arg Gly Leu Asn Arg Leu Phe Ala Ser Thr Ser Thr Met Gly Glu 50 60

Val Ala Asp Ala Gly Met Asp Ala Val Gln Lys Arg Leu Met Phe Asp 65 70 75 80

Asp Glu Cys Ile Leu Val Asp Glu Asn Asp Lys Val Val Gly Tyr Asp 85 90 95

Ser Lys Tyr Asn Cys His Leu Met Glu Lys Ile Glu Ala Glu Asn Leu 100 105 110

Leu His Arg Ala Phe Ser Val Phe Leu Phe Asn Ser Lys Tyr Glu Leu 115 120 125

Leu Leu Gln Gln Arg Ser Ala Thr Lys Val Thr Phe Pro Leu Val Trp 130 135 140

Thr Asn Thr Cys Cys Ser His Pro Leu Phe Arg Asp Ser Glu Leu Ile 145 150 155 160

Glu Glu Asn Phe Leu Gly Val Arg Asn Ala Ala Gln Arg Lys Leu Leu 165 170 175

Asp Glu Leu Gly Ile Pro Ala Glu Asp Val Pro Val Asp Glu Phe Thr 180 185 190

Pro Leu Gly Arg Ile Leu Tyr Lys Ala Pro Ser Asp Gly Lys Trp Gly 195 200 205

Glu His Glu Leu Asp Tyr Leu Leu Phe Ile Val Arg Asp Val Lys Tyr 210 215 220

Asp Pro Asn Pro Asp Glu Val Ala Asp Ala Lys Tyr Val Asn Arg Glu 225 230 235 240

Glu Leu Lys Glu Ile Leu Arg Lys Ala Asp Ala Gly Glu Gly Ile 245 250 255

Lys Leu Ser Pro Trp Phe Arg Leu Val Val Asp Asn Phe Leu Phe Lys

260

265

270

Trp Trp Asp His Val Glu Glu Gly Lys Ile Lys Asp Val Ala Asp Met 275 280 285

Lys Thr Ile His Lys Leu Thr 290 295

<210> 40

<211> 234

<212> PRT

<213> Adonis Palaestina

<400> 40

Met Gly Glu Val Thr Asp Ala Gly Met Asp Ala Val Gln Lys Arg Leu  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Met Phe Asp Asp Glu Cys Ile Leu Val Asp Glu Asn Asp Lys Val Val 20 25 30

Gly His Asp Ser Lys Tyr Asn Cys His Leu Met Glu Lys Ile Glu Ala 35 40 45

Glu Asn Leu Leu His Arg Ala Phe Ser Val Phe Leu Phe Asn Ser Lys
50 55 60

Tyr Glu Leu Leu Gln Gln Arg Ser Ala Thr Lys Val Thr Phe Pro 65 70 75 80

Leu Val Trp Thr Asn Thr Cys Cys Ser His Pro Leu Phe Arg Asp Ser 85 90 95

Glu Leu Ile Glu Glu Asn Tyr Leu Gly Val Arg Asn Ala Ala Gln Arg 100 105 110

Lys Leu Leu Asp Glu Leu Gly Ile Pro Ala Glu Asp Val Pro Val Asp 115 120 125

Glu Phe Thr Pro Leu Gly Arg Ile Leu Tyr Lys Ala Pro Ser Asp Gly 130 135

Lys Trp Gly Glu His Glu Leu Asp Tyr Leu Leu Phe Ile Val Arg Asp 145 150 155 160

Val Lys Tyr Asp Pro Asn Pro Asp Glu Val Ala Asp Ala Lys Tyr Val 165 170 175

Asn Arg Glu Glu Leu Arg Glu Ile Leu Arg Lys Ala Asp Ala Gly Glu 180 185 190

Glu Gly Leu Lys Leu Ser Pro Trp Phe Arg Leu Val Val Asp Asn Phe 195 200 205

Leu Phe Lys Trp Trp Asp His Val Glu Gln Gly Thr Ile Lys Glu Val 210 215 220

Ala Asp Met Lys Thr Ile His Lys Leu Thr 225 230

<210> 41 <211> 238

<212> PRT <213> Oryza Sativa

<400> 41

Met Ala Gly Ala Ala Ala Val Glu Asp Ala Gly Met Asp Glu Val 1 5 10 15

Gln Lys Arg Leu Met Phe Asp Asp Glu Cys Ile Leu Val Asp Glu Gln 20 25 30

Asp Asn Val Val Gly His Glu Ser Lys Tyr Asn Cys His Leu Met Glu 35 40 45

Lys Ile Glu Ser Glu Asn Leu Leu His Arg Ala Phe Ser Val Phe Leu 50 60

Phe Asn Ser Lys Tyr Glu Leu Leu Leu Gln Gln Arg Ser Ala Thr Lys 65 70 75 80

Val Thr Phe Pro Leu Val Trp Thr Asn Thr Cys Cys Ser His Pro Leu 85 90 95

Tyr Arg Glu Ser Glu Leu Ile Gln Glu Asn Tyr Leu Gly Val Arg Asn 100 105 110

Ala Ala Gln Arg Lys Leu Leu Asp Glu Leu Gly Ile Pro Ala Glu Asp 115 120 125

Val Pro Val Asp Gln Phe Thr Pro Leu Gly Arg Met Leu Tyr Lys Ala 130 135 140

Pro Ser Asp Gly Lys Trp Gly Glu His Glu Leu Asp Tyr Leu Leu Phe 145 150 155 160

Ile Val Arg Asp Val Lys Val Val Pro Asn Pro Asp Glu Val Ala Asp 165 170 175

Val Lys Tyr Val Ser Arg Glu Gln Leu Lys Glu Leu Ile Arg Lys Ala 180 185 190

Asp Ala Gly Glu Glu Gly Leu Lys Leu Ser Pro Trp Phe Arg Leu Val 195 200 205

Val Asp Asn Phe Leu Met Gly Trp Trp Asp His Val Glu Lys Gly Thr 210 215 220

Leu Asn Glu Ala Val Asp Met Glu Thr Ile His Lys Leu Lys 225 230 235

<210> 42

<211> 233

<212> PRT

<213> Arabidopsis thaliana

<400> 42

Met Thr Asp Ser Asn Asp Ala Gly Met Asp Ala Val Gln Arg Arg Leu  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Met Phe Glu Asp Glu Cys Ile Leu Val Asp Glu Asn Asn Arg Val Val 20 25 30

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Gly His Asp Thr Lys Tyr Asn Cys His Leu Met Glu Lys Ile Glu Ala Glu Asn Leu Leu His Arg Ala Phe Ser Val Phe Leu Phe Asn Ser Lys Tyr Glu Leu Leu Gln Gln Arg Ser Lys Thr Lys Val Thr Phe Pro Leu Val Trp Thr Asn Thr Cys Cys Ser His Pro Leu Tyr Arg Glu Ser Glu Leu Ile Glu Glu Asn Val Leu Gly Val Arg Asn Ala Ala Gln Arg Lys Leu Phe Asp Glu Leu Gly Ile Val Ala Glu Asp Val Pro Val Asp Glu Phe Thr Pro Leu Gly Arg Met Leu Tyr Lys Ala Pro Ser Asp Gly 135 Lys Trp Gly Glu His Glu Val Asp Tyr Leu Leu Phe Ile Val Arg Asp 145 150 155 Val Lys Leu Gln Pro Asn Pro Asp Glu Val Ala Glu Ile Lys Tyr Val 165 Ser Arg Glu Glu Leu Lys Glu Leu Val Lys Lys Ala Asp Ala Gly Asp Glu Ala Val Lys Leu Ser Pro Trp Phe Arg Leu Val Val Asp Asn Phe 200 Leu Met Lys Trp Trp Asp His Val Glu Lys Gly Thr Ile Thr Glu Ala 210 215 Ala Asp Met Lys Thr Ile His Lys Leu <210> 43 <211> 293 <212> PRT <213> Haematococcus pluvialis <400> 43

Met Leu Arg Ser Leu Leu Arg Gly Leu Thr His Ile Pro Arg Val Asn

Ser Ala Gln Gln Pro Ser Cys Ala His Ala Arg Leu Gln Phe Lys Leu

Arg Ser Met Gln Leu Leu Ser Glu Asp Arg Thr Asp His Met Arg Gly

Ala Ser Thr Trp Ala Gly Gly Gln Ser Gln Asp Glu Leu Met Leu Lys

Asp Glu Cys Ile Leu Val Asp Val Glu Asp Asn Ile Thr Gly His Ala

Ser Lys Leu Glu Cys His Lys Phe Leu Pro His Gln Pro Ala Gly Leu

90 95

Leu His Arg Ala Phe Ser Val Phe Leu Phe Asp Asp Gln Gly Arg Leu 100 105 110

Leu Leu Gln Gln Arg Ala Arg Ser Lys Ile Thr Phe Pro Ser Val Trp
115 120 125

Thr Asn Thr Cys Cys Ser His Pro Leu His Gly Gln Thr Pro Asp Glu 130 135 140

Val Asp Gln Leu Ser Gln Val Ala Asp Gly Thr Val Pro Gly Ala Lys 145 150 155 160

Ala Ala Ala Ile Arg Lys Leu Glu His Glu Leu Gly Ile Pro Ala His 165 170 175

Gln Leu Pro Ala Ser Ala Phe Arg Phe Leu Thr Arg Leu His Tyr Cys 180 185 190

Ala Ala Asp Val Gln Pro Ala Ala Thr Gln Ser Ala Leu Trp Gly Glu 195 200 205

His Glu Met Asp Tyr Ile Leu Phe Ile Arg Ala Asn Val Thr Leu Ala 210 215 220

Pro Asn Pro Asp Glu Val Asp Glu Val Arg Tyr Val Thr Gln Glu Glu 225 230 235 240

Leu Arg Gln Met Met Gln Pro Asp Asn Gly Leu Gln Trp Ser Pro Trp 245 250 255

Phe Arg Ile Ile Ala Ala Arg Phe Leu Glu Arg Trp Trp Alá Asp Leu 260 265 270

Asp Ala Ala Leu Asn Thr Asp Lys His Glu Asp Trp Gly Thr Val His 275 280 285

His Ile Asn Glu Ala 290

<210> 44

<211> 304

<212> PRT

<213> Haematococcus pluvialis

<400> 44

Met Leu Arg Ser Leu Leu Arg Gly Leu Thr His Ile Pro Arg Val Asn
1 5 10 15

Ser Ala Gln Gln Pro Ser Cys Ala His Ala Arg Leu Gln Phe Lys Leu 20 25 30

Arg Ser Met Gln Met Thr Leu Met Gln Pro Ser Ile Ser Ala Asn Leu  $35 \hspace{1cm} 40 \hspace{1cm} 45$ 

Ser Arg Ala Glu Asp Arg Thr Asp His Met Arg Gly Ala Ser Thr Trp 50 55 60

Ala Gly Gly Gln Ser Gln Asp Glu Leu Met Leu Lys Asp Glu Cys Ile 65 70 75 80



Leu Val Asp Val Glu Asp Asn Ile Thr Gly His Ala Ser Lys Leu Glu Cys His Lys Phe Leu Pro His Pro Ala Gly Leu Leu His Arg Ala Phe 105 Ser Val Phe Leu Phe Asp Asp Gln Gly Arg Leu Leu Leu Gln Gln Arg Ala Arg Ser Lys Ile Thr Phe Pro Ser Val Trp Thr Asn Thr Cys Cys 135 Ser His Pro Leu His Gly Gln Thr Pro Asp Glu Val Asp Gln Leu Ser Gln Val Ala Asp Gly Thr Val Pro Gly Ala Lys Ala Ala Ala Ile Arg 170 Lys Leu Glu His Glu Leu Gly Ile Pro Ala His Gln Leu Pro Ala Ser 185 Ala Phe Arg Phe Leu Thr Arg Leu His Tyr Cys Ala Ala Asp Val Gln Pro Ala Ala Thr Gln Ser Ala Leu Trp Gly Glu His Glu Met Asp Tyr 215 Ile Leu Phe Ile Arg Ala Asn Val Thr Leu Ala Pro Asn Pro Asp Glu 235 Val Asp Glu Val Arg Tyr Val Thr Gln Glu Glu Leu Arg Gln Met Met Gln Pro Asp Asn Gly Leu Gln Trp Ser Pro Trp Phe Arg Ile Ile Ala 265 Ala Arg Phe Leu Glu Arg Trp Trp Ala Asp Leu Asp Ala Ala Leu Asn Thr Asp Lys His Glu Asp Trp Gly Thr Val His His Ile Asn Glu Ala 290 <210> 45 <211> 307 <212> PRT <213> Chlamydomonas reinhardtii <400> 45 Met Arg Ser Ser Phe Ile Glu Pro Lys Pro Arg Ala Gln Pro Val Leu Ser Arg Gly Arg Ala Ser Met Arg Leu Ala Gln Ser Arg Ala Leu Val

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65					70					75					80		
Asp	Glu	Gln	Asp	Arg 85	Leu	Leu	Gly	Thr	Ala 90	Asn	Lys	Tyr	Asp	Cys 95	His		
Arg	Phe	Glu	Ala 100	Ala	Lys	Gly	Gln	Pro 105	Cys	Gly	Arg	Leu	His 110	Arg	Ala		
Phe	Ser	Val 115	Phe	Leu	Phe	Ser	Pro 120	Asp	Gly	Arg	Leu	Leu 125	Leu	Gln	Gln		
Arg	Ala 130	Ala	Ser	Lys	Val	Thr 135	Phe	Pro	Gly	Val	Trp 140	Thr	Asn	Thr	Cys		
Cys 145	Ser	His	Pro	Leu	Ala 150	Gly	Gln	Ala	Pro	Asp 155	Glu	Val	Asp	Leu	Pro 160		
Ala	Ala	Val	Ala	Ser 165	Gly	Gln	Val	Pro	Gly 170	Ile	Lys	Ala	Ala	Ala 175	Val		
Arg	Lys	Leu	Gln 180	His	Glu	Leu	Gly	Ile 185	Pro	Pro	Glu	Gln	Val 190	Pro	Ala		
Ser	Ser	Phe 195	Ser	Phe	Leu	Thr	Arg 200	Leu	His	Tyr	Cys	Ala 205	Ala	Asp	Thr		
Ala	Thr 210	His	Gly	Pro	Ala	Ala 215	Glu	Trp	Gly	Glu	His 220	Glu	Val	Asp	Tyr		
Val 225		Phe	Val	Arg	Pro 230	Gln	Gln	Pro	Val	Ser 235	Leu	Gln	Pro	Asn	Pro 240		
Asp	Glu	Val	Asp	Ala 245	Thr	Arg	Tyr	Val	Thr 250	Leu	Pro	Glu	Leu	Gln 255	Ser		
Met	Met	Ala	Asp 260		Gly	Leu	Ser	Trp 265	Ser	Pro	Trp	Phe	Arg 270	Ile	e Leu		
Ala	Thr	Gln 275		Ala	Phe	. Leu	Pro 280	Ala	Trp	Trp	Gly	/ Asp 285	Leu S	Lys	arg Arg		
Arg	Trp 290		Pro	Gly	/ Gly	Ser 295	Arg	g Lev	ı Ser	Asp	300	Gly	/ Thr	Ile	e His		
Arg 305		. Met	:														
<21	.0> 4	1848															
	L2> I L3> <i>I</i>		is pa	alae	stina	ā											
<40 GA0	00> G GAGA	46 AAAA	GAG'	TGTT	ATA '	TTAA'	rgtt.	AC T	GTCG	CATT	с тт	GCAA	CACA	TAT'	TCAGACT	6	0
CC	TTT?	TCTT	GTT	ттст	CTT	CAAA	ACAA	CA A	ACTA	ATGT	G AC	GGAG	TATC	TAG	CTATGGA	12	0
AC'	ract'	TGGT	GTT	CGCA	ACC	TCAT	CTCT	тс т	TGCC	CTGT	C TG	GACT	TTTG	GAA	CAAGAAA	18	0
CC'	TTAG	TAGT	TCA	AAAC	TAG	CTTA	TAAC	AT A	CATC	GATA	T GG	TTCT	TCTT	GTA	GAGTAGA	24	0



	110 77/0	5000					
,	TTTTCAAGTG	AGGGCTGATG	GTGGAAGCGG	GAGTAGAACT	TCTGTTGCTT	ATAAAGAGGG	300
,	TTTTGTGGAC	GAGGAGGATT	TTATCAAAGC	TGGTGGTTCT	GAGCTTTTGT	TTGTCCAAAT	360
(	GCAGCAAACA	AAGTCTATGG	AGAAACAGGC	CAAGCTCGCC	GATAAGTTGC	CACCAATACC	420
	TTTCGGAGAA	TCTGTGATGG	ACTTGGTTGT	AATAGGTTGT	GGACCTGCTG	GTCTTTCACT	480
,	GGCTGCAGAA	GCTGCTAAGC	TAGGCTTGAA	AGTTGGCCTT	ATTGGTCCTG	ATCTTCCTTT	540
	TACAAATAAT	TATGGTGTGT	GGGAAGACGA	GTTCAAAGAT	CTTGGACTTG	AACGTTGTAT	600
	CGAGCATGCT	TGGAAGGACA	CCATCGTATA	TCTTGACAAT	GATGCTCCTG	TCCTTATTGG	660
	TCGTGCATAT	GGACGAGTTA	GCCGGCATTT	GCTGCATGAA	GAGTTGCTGA	AAAGGTGTGT	720
	CGAGTCAGGT	GTATCATATC	TGAATTCTAA	AGTGGAAAGG	ATCACTGAAG	CTGGTGATGG	780
	CCATAGTCTT	GTAGTTTGTG	AAAACGACAT	CTTTATCCCT	TGCAGGCTTG	CTACTGTTGC	840
	ATCTGGAGCA	GCTTCAGGGA	AACTTTTGGA	GTATGAAGTA	GGTGGCCCTC	GTGTTTGTGT	900
	CCAAACTGCT	TATGGTGTGG	AGGTTGAGGT	GGAGAACAAT	CCATACGATC	CCAACTTAAT	960
	GGTATTTATG	GACTACAGAG	ACTATATGCA	ACAGAAATTA	CAGTGCTCGG	AAGAAGAATA	1020
	TCCAACATTT	CTCTATGTCA	TGCCCATGTC	GCCAACAAGA	CTTTTTTTG	AGGAAACCTG	1080
	TTTGGCCTCA	AAAGATGCCA	TGCCTTTCGA	TCTACTGAAG	AGAAAACTAA	TGTCACGATT	1140
	GAAGACTCTG	GGTATCCAAG	TTACAAAAAT	TTATGAAGAG	GAATGGTCTT	ATATTCCTGT	1200
	TGGGGGTTCT	TTACCAAACA	CAGAGCAAAA	GAACCTAGCA	TTTGGTGCTG	CAGCAAGCAT	1260
	GGTGCATCCA	GCAACAGGCT	ATTCGGTTGT	ACGATCACTA	TCAGAAGCTC	CAAAATATGC	1320
	TTCTGTAATT	GCAAAGATTT	TGAAGCAAGA	TAACTCTGCA	TATGTGGTT	CTGGACAAAG	1380
	CAGTGCAGTA	AACATTTCAA	TGCAAGCATG	GAGCAGTCTT	TGGCCAAAG	AGCGAAAACG	1440
	TCAAAGAGCA	TTCTTTCTT	TCGGGTTAG	A GCTTATTGT	CAGCTAGAT!	A TTGAAGCAAC	1500
	CAGAACGTTC	TTTAGAACCI	TCTTCCGCTT	GCCAACTTGG	ATGTGGTGG	GTTTCCTTGG	1560
	GTCTTCACTA	A TCATCTTTC	ATCTTGTAT	GTTTTCCATO	TACATGTTT	TTTTGGCCCC	1620
	GAACAGCATO	G AGGATGTCA	C TTGTGAGACA	A TTTGCTTTC	A GATCCTTCT	G GTGCAGTTAT	1680
	GGTTAAAGC	TACCTCGAA	A GGTAATCTG	r TTTATGAAA	C TATAGTGTC	г саттааатаа	1740
						r TCTAATCTCA	
				AAAAAAAA A			1848

<sup>&</sup>lt;210> 47

<sup>&</sup>lt;211> 529

<sup>&</sup>lt;212> PRT

<sup>. &</sup>lt;213> Adonis palaestina

Met Glu Leu Leu Gly Val Arg Asn Leu Ile Ser Ser Cys Pro Val Trp

1					5					10					15	
Thr	Phe	Gly	Thr 20		rg A	Asn :	Leu	Ser	Ser 25	Ser	Lys	Leu	Ala	Tyr 30	Asn	Ile
His	Arg	Tyr 35	GJŻ	, S	er S	Ser	Cys	Arg 40	Val	Asp	Phe	Gln	Val 45	Arg	Ala	Asp
Gly	Gly 50	Ser	Gly	, S	er A	Arg	Ser 55	Ser	Val	Ala	Tyr	Lys 60	Glu	Gly	Phe	Val
Asp 65	Glu	Glu	Asp	P	he :	lle 70	Lys	Ala	Gly	Gly	Ser 75	Glu	Leu	Leu	Phe	Val 80
Gln	Met	Gln	Gli		hr ! 85	Lys	Ser	Met	Glu	Lys 90	Gln	Ala	Lys	Leu	Ala 95	Asp
Lys	Leu	Pro	Pro		le	Pro	Phe	Gly	Glu 105	Ser	Val	Met	Asp	Leu 110	Val	Val
Ile	Gly	Cys 115		y F	ro.	Ala	Gly	Leu 120	Ser	Leu	Ala	Ala	Glu 125	Ala	Ala	Lys
Leu	Gly 130		Ly	s V	/al	Gly	Leu 135	Ile	Gly	Pro	Asp	Leu 140	Pro	Phe	Thr	Asn
Asn 145	Tyr	Gly	v Va	1 1	ſrp	Glu 150	Asp	Glu	Phe	Lys	Asp 155	Leu	Gly	Leu	Glu	Arg 160
Cys	Ile	Glu	ı Hi		Ala 165	Trp	Lys	Asp	Thr	Ile 170	Val	Туг	Leu	Asp	Asn 175	Asp
Ala	Pro	Val	L Le	u :	Ile	Gly	Arg	Ala	Tyr 185	Gly	Arç	y Val	Ser	Arg 190	His	Leu
Leu	His	Gl: 19:		u I	Leu	Leu	Lys	Arg 200	Cys	Val	Glu	ı Ser	Gly 205	/ Val	Ser	Tyr
Leu	Asp 210		r Lj	's	Val	Glu	Arg 215	Ile	Thr	Glu	ı Ala	a Gly 220	y Asp O	o Gly	/ His	Ser
Leu 225		L Va	1 C	/S	Glu	Asn 230	Glu	ılle	Phe	e Ile	e Pro 23	o Cys	s Ar	g Leu	ı Ala	Thr 240
Va]	Ala	a Se	r G	Ly	Ala 245	Ala	Ser	Gly	/ Lys	25	u Lei 0	u Gl	u Ty:	r Glı	val 255	Gly
Gly	y Pro	o Ar		al 60	Cys	Val	Glr	n Thr	Ala 265	а Ту 5	r Gl	y Va	l Gl	u Vai 270	l Gli	ı Val
Gli	u As	n As 27		ro	Tyr	Asp	Pro	280	n Lei	ı Me	t Va	l Ph	e Me 28	t Ası 5	o Ty:	r Arg
As	р Ту 29		et G	ln	Gln	Lys	Le: 29	u Glı 5	n Cy	s Se	r Gl	u Gl 30	u Gl O	и Ту	r Pr	o Thr
Ph 30		u Ty	yr V	al	Met	Pro	Me	t Se	r Pr	o Th	r Ar 31	g Le 5	u Ph	e Ph	e Gl	u Glu 320
Th	r Cy	s Le	eu A	la	Ser 325	Ly:	s As	p Al	a Me	t Pr 33	o Ph	e As	sp Le	u Le	u Ly 33	s Arç 5

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Lys Leu Met Ser Arg Leu Lys Thr Leu Gly Ile Gln Val Thr Lys Val 345

Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn 365

Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His 370

Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys 390

Tyr Ala Ser Val Ile Ala Lys Ile Leu Lys Gln Asp Asn Ser Ala Tyr

Val Val Ser Gly Gln Ser Ser Ala Val Asn Ile Ser Met Gln Ala Trp
420 425 430

Ser Ser Leu Trp Pro Lys Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu 435 440 445

Phe Gly Leu Glu Leu Ile Val Gln Leu Asp Ile Glu Ala Thr Arg Thr 450 455 460

Phe Phe Arg Thr Phe Phe Arg Leu Pro Thr Trp Met Trp Trp Gly Phe 465 470 475 480

Leu Gly Ser Ser Leu Ser Ser Phe Asp Leu Val Leu Phe Ser Met Tyr 485 490 495

Met Phe Val Leu Ala Pro Asn Ser Met Arg Met Ser Leu Val Arg His 500 505 510

Leu Leu Ser Asp Pro Ser Gly Ala Val Met Val Arg Ala Tyr Leu Glu 515 520 525

Arg

<210> 48

<211> 378

<212> PRT

<213> Potato

<400> 48

Asp Glu Phe Lys Asp Leu Gly Leu Gln Ala Cys Ile Glu His Val Trp

1 5 10 15

Arg Asp Thr Ile Val Tyr Leu Asp Asp Asp Pro Ile Leu Ile Gly 20 25 30

Arg Ala Tyr Gly Arg Val Ser Arg His Leu Leu His Glu Glu Leu Leu 35 40 45

Lys Arg Cys Val Glu Ala Gly Val Leu Tyr Leu Asn Ser Lys Val Asp 50 60

Arg Ile Val Glu Ala Thr Asn Gly His Ser Leu Val Glu Cys Glu Gly 65 70 75 80

Asp Val Val Ile Pro Cys Arg Phe Val Thr Val Ala Ser Gly Ala Ala 85 90 95 Ser Gly Lys Phe Leu Gln Tyr Glu Leu Gly Gly Pro Arg Val Ser Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Asp Asn Asn Pro Phe Asp 120 Pro Ser Leu Met Val Phe Met Asp Tyr Arg Asp Tyr Val Arg His Asp 135 Ala Gln Ser Leu Glu Ala Lys Tyr Pro Thr Phe Leu Tyr Ala Met Pro Met Ser Pro Thr Arg Val Phe Phe Glu Glu Thr Cys Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Lys Leu Met Leu Arg Leu 185 Asn Thr Leu Gly Val Arg Ile Lys Glu Ile Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln Lys Thr Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser 235 Val Val Arg Ser Leu Ser Glu Ala Pro Lys Cys Ala Phe Val Leu Ala 245 Asn Ile Leu Arg Gln Asn His Ser Lys Asn Met Leu Thr Ser Ser Ser 265 Thr Pro Ser Ile Ser Thr Gln Ala Trp Asn Thr Leu Trp Pro Gln Glu Arg Lys Arg Gln Arg Ser Phe Phe Leu Phe Gly Leu Ala Leu Ile Leu

Arg Lys Arg Gin Arg Ser Phe Phe Led Phe Gly Led Ala Led Tie Led 290 295 300

Gln Leu Asp Ile Glu Gly Ile Arg Ser Phe Phe Arg Ala Phe Phe Arg 305 310 315

Val Pro Lys Trp Met Trp Gln Gly Phe Leu Gly Ser Ser Leu Ser Xaa 325 330 335

Ala Asp Leu Met Leu Phe Ala Phe Tyr Met Phe Ile Ile Ala Pro Asn 340 345 350

Asp Met Arg Arg Gly Leu Ile Arg His Leu Leu Ser Asp Pro Thr Gly 355 360 365

Ala Thr Leu Ile Arg Thr Tyr Leu Thr Phe 370 375

<210> 49

<211> 524

<212> PRT

<213> Arabidopsis thaliana

<400> 49

Met Glu Cys Val Gly Ala Arg Asn Phe Ala Ala Met Ala Val Ser Thr Phe Pro Ser Trp Ser Cys Arg Arg Lys Phe Pro Val Val Lys Arg Tyr Ser Tyr Arg Asn Ile Arg Phe Gly Leu Cys Ser Val Arg Ala Ser Gly Gly Gly Ser Ser Gly Ser Glu Ser Cys Val Ala Val Arg Glu Asp Phe Ala Asp Glu Glu Asp Phe Val Lys Ala Gly Gly Ser Glu Ile Leu Phe Val Gln Met Gln Gln Asn Lys Asp Met Asp Glu Gln Ser Lys Leu Val Asp Lys Leu Pro Pro Ile Ser Ile Gly Asp Gly Ala Leu Asp His Val Val Ile Gly Cys Gly Pro Ala Gly Leu Ala Leu Ala Ala Glu Ser Ala 120 Lys Leu Gly Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Glu Asp Glu Phe Asn Asp Leu Gly Leu Gln 155 Lys Cys Ile Glu His Val Trp Arg Glu Thr Ile Val Tyr Leu Asp Asp Asp Lys Pro Ile Thr Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg Arg Leu Leu His Glu Glu Leu Leu Arg Arg Cys Val Glu Ser Gly Val Ser Tyr Leu Ser Ser Lys Val Asp Ser Ile Thr Glu Ala Ser Asp Gly Leu Arg Leu Val Ala Cys Asp Asp Asn Asn Val Ile Pro Cys Arg Leu Ala 235 Thr Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Gln Tyr Glu Val Gly Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu Asn Ser Pro Tyr Asp Pro Asp Gln Met Val Phe Met Asp Tyr Arg Asp Tyr Thr Asn Glu Lys Val Arg Ser Leu Glu Ala Glu Tyr Pro 295 Thr Phe Leu Tyr Ala Met Pro Met Thr Lys Ser Arg Leu Phe Phe Glu Glu Thr Cys Leu Ala Ser Lys Asp Val Met Pro Phe Asp Leu Leu Lys 325

330

335

Thr Lys Leu Met Jeu Arg Leu Asp Thr Leu Gly Ile Arg Ile Leu Lys 350

Thr Tyr Glu Glu Glu Trp Ser Tyr 360 Ile Pro Val Gly Gly Ser Leu Pro 350

Asn Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val 370

His Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro 385 390 395 400

Lys Tyr Ala Ser Val Ile Ala Glu Ile Leu Arg Glu Glu Thr Thr Lys 405 410 415

Gln Ile Asn Ser Asn Ile Ser Arg Gln Ala Trp Asp Thr Leu Trp Pro 420 425 430

Pro Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu Phe Gly Leu Ala Leu 435 440 445

Ile Val Gln Phe Asp Thr Glu Gly Ile Arg Ser Phe Phe Arg Thr Phe 450 460

Phe Arg Leu Pro Lys Trp Met Trp Gln Gly Phe Leu Gly Ser Thr Leu 465 470 475 480

Thr Ser Gly Asp Leu Val Leu Phe Ala Leu Tyr Met Phe Val Ile Ser 485 490 495

Pro Asn Asn Leu Arg Lys Gly Leu Ile Asn His Leu Ile Ser Asp Pro 500 505 510

Thr Gly Ala Thr Met Ile Lys Thr Tyr Leu Lys Val 515 520

<210> 50

<211> 529

<212> PRT

<213> Adonis palaestina

<400> 50

Met Glu Leu Leu Gly Val Arg Asn Leu Ile Ser Ser Cys Pro Val Trp
1 5 10 15

Thr Phe Gly Thr Arg Asn Leu Ser Ser Ser Lys Leu Ala Tyr Asn Ile 20 25 30

His Arg Tyr Gly Ser Ser Cys Arg Val Asp Phe Gln Val Arg Ala Asp 35 40 45

Gly Gly Ser Gly Ser Arg Ser Ser Val Ala Tyr Lys Glu Gly Phe Val 50 55 60

Asp Glu Glu Asp Phe Ile Lys Ala Gly Gly Ser Glu Leu Leu Phe Val 65 70 75 80

Gln Met Gln Gln Thr Lys Ser Met Glu Lys Gln Ala Lys Leu Ala Asp

85 90 95

Lys Leu Pro Pro Ile Pro Phe Gly Glu Ser Val Met Asp Leu Val Val 105 Ile Gly Cys Gly Pro Ala Gly Leu Ser Leu Ala Ala Glu Ala Ala Lys Leu Gly Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Glu Arg 150 Cys Ile Glu His Ala Trp Lys Asp Thr Ile Val Tyr Leu Asp Asn Asp Ala Pro Val Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu Leu His Glu Glu Leu Leu Lys Arg Cys Val Glu Ser Gly Val Ser Tyr Leu Asp Ser Lys Val Glu Arg Ile Thr Glu Ala Gly Asp Gly His Ser Leu Val Val Cys Glu Asn Glu Ile Phe Ile Pro Cys Arg Leu Ala Thr 230 Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Glu Tyr Glu Val Gly Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu Asn Asn Pro Tyr Asp Pro Asn Leu Met Val Phe Met Asp Tyr Arg Asp Tyr Met Gln Gln Lys Leu Gln Cys Ser Glu Glu Glu Tyr Pro Thr Phe Leu Tyr Val Met Pro Met Ser Pro Thr Arg Leu Phe Phe Glu Glu Thr Cys Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Arg 330 Lys Leu Met Ser Arg Leu Lys Thr Leu Gly Ile Gln Val Thr Lys Val Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys Tyr Ala Ser Val Ile Ala Lys Ile Leu Lys Gln Asp Asn Ser Ala Tyr 405 410

Val Val Ser Gly Gln Ser Ser Ala Val Asn Ile Ser Met Gln Ala Trp 420 425 430

Ser Ser Leu Trp Pro Lys Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu 435 440 445

Phe Gly Leu Glu Leu Ile Val Gln Leu Asp Ile Glu Ala Thr Arg Thr 450 455 460

Phe Phe Arg Thr Phe Phe Arg Leu Pro Thr Trp Met Trp Trp Gly Phe 465 470 475 480

Leu Gly Ser Ser Leu Ser Ser Phe Asp Leu Val Leu Phe Ser Met Tyr
485 490 495

Met Phe Val Leu Ala Pro Asn Ser Met Arg Met Ser Leu Val Arg His 500 505 510

Leu Leu Ser Asp Pro Ser Gly Ala Val Met Val Arg Ala Tyr Leu Glu 515 520 525

Arg

<210> 51

<211> 529

<212> PRT

<213> Adonis palaestina

<400> 51

Met Glu Leu Leu Gly Val Arg Asn Leu Ile Ser Ser Cys Pro Val Trp
1 5 10 15

Thr Phe Gly Thr Arg Asn Leu Ser Ser Ser Lys Leu Ala Tyr Asn Ile 20 25 30

His Arg Tyr Gly Ser Ser Cys Arg Val Asp Phe Gln Val Arg Ala Asp 35 40 45

Gly Gly Ser Gly Ser Arg Thr Ser Val Ala Tyr Lys Glu Gly Phe Val
50 60

Asp Glu Glu Asp Phe Ile Lys Ala Gly Gly Ser Glu Leu Leu Phe Val 65 70 75 80

Gln Met Gln Gln Thr Lys Ser Met Glu Lys Gln Ala Lys Leu Ala Asp 85 90 95

Lys Leu Pro Pro Ile Pro Phe Gly Glu Ser Val Met Asp Leu Val Val 100 105 110

Ile Gly Cys Gly Pro Ala Gly Leu Ser Leu Ala Ala Glu Ala Ala Lys 115 120 125

Leu Gly Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn 130 135 140

Asn Tyr Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Glu Arg 145 150 155 160



Cys	Ile	Glu	His	Ala 165	Trp	Lys	Asp	Thr	Ile 170	Val	Tyr	Leu	Asp	Asn 175	Asp
Ala	Pro	Val	Leu 180	Ile	Gly	Arg	Ala	Tyr 185	Gly	Arg	Val	Ser	Arg 190	His	Leu
Leu	His	Glu 195	Glu	Leu	Leu	Lys	Arg 200	Cys	Val	Glu	Ser	Gly 205	Val	Ser	Tyr
Leu	Asn 210	Ser	Lys	Val	Glu	Arg 215	Ile	Thr	Glu	Ala	Gly 220	Asp	Gly	His	Ser
Leu 225	Val	Val	Cys	Glu	Asn 230	Asp	Ile	Phe	Ile	Pro 235	Cys	Arg	Leu	Ala	Thr 240
Val	Ala	Ser	Gly	Ala 245	Ala	Ser	Gly	Lys	Leu 250	Leu	Glu	Tyr	Glu	Val 255	Gly
Gly	Pro	Arg	Val 260	Cys	Val	Gln	Thr	Ala 265	Tyr	Gly	Val	Glu	Val 270	Glu	Val
Glu	Asn	Asn 275	Pro	Tyr	Asp	Pro	Asn 280	Leu	Met	Val	Phe	Met 285	Asp	Tyr	Arg
Asp	Tyr 290	Met	Gln	Gln	Lys	Leu 295	Gln	Cys	Ser	Glu	Glu 300	Glu	Tyr	Pro	Thr
Phe 305	Leu	Tyr	Val	Met	Pro 310	Met	Ser	Pro	Thr	Arg 315	Leu	Phe	Phe	Glu	Glu 320
Thr	Cys	Leu	Ala	Ser 325	Lys	Asp	Ala	Met	Pro 330	Phe	Asp	Leu	Leu	Lys 335	Arg
Lys	Leu	Met	Ser 340	Arg	Leu	Lys	Thr	Leu 345	Gly	Ile	Gln	Val	Thr 350	Lys	Ile
Tyr	Glu	Glu 355	Glu	Trp	Ser	Tyr	Ile 360	Pro	Val	Gly	Gly	Ser 365	Leu	Pro	Asn
Thr	Glu 370	Gln	Lys	Asn	Leu	Ala 375	Phe	Gly	Ala	Ala	Ala 380	Ser	Met	Val	His
Pro 385	Ala	Thr	Gly	Tyr	Ser 390		Val	Arg	Ser	Leu 395		Glu	Ala		Lys 400
Tyr	Ala	Ser	Val	Ile 405	Ala	Lys	Ile	Leu	Lys 410	Gln	Asp	Asn	Ser	Ala 415	Tyr
Val	Val	Ser	Gly 420	Gln	Ser	Ser	Ala	Val 425	Asn	Ile	Ser	Met	Gln 430	Ala	Trp
Ser	Ser	Leu 435	Trp	Pro	Lys	Glu	Arg 440	Lys	Arg	Gln	Arg	Ala 445	Phe	Phe	Leu
Phe	Gly 450	Leu	Glu	Leu	Ile	Val 455	Gln	Leu	Asp	Ile	Glu 460	Ala	Thr	Arg	Thr
Phe 465	Phe	Arg	Thr	Phe	Phe 470	Arg	Leu	Pro	Thr	Trp 475	Met	Trp	Trp	Gly	Phe 480
Leu	Gly	Ser	Ser	Leu	Ser	Ser	Phe	Asp	Leu	Val	Leu	Phe	Ser	Met	Tyr

495

485 490

Met Phe Val Leu Ala Pro Asn Ser Met Arg Met Ser Leu Val Arg His 500 505 510

Leu Leu Ser Asp Pro Ser Gly Ala Val Met Val Lys Ala Tyr Leu Glu 515 520 525

Arg

<210> 52

<211> 533

<212> PRT

<213> Lettuce

<400> 52

Met Glu Cys Phe Gly Ala Arg Asn Met Thr Ala Thr Met Ala Val Phe  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Thr Cys Pro Arg Phe Thr Asp Cys Asn Ile Arg His Lys Phe Ser Leu 20 25 30

Leu Lys Gln Arg Arg Phe Thr Asn Leu Ser Ala Ser Ser Ser Leu Arg 35 40 45

Gln Ile Lys Cys Ser Ala Lys Ser Asp Arg Cys Val Val Asp Lys Gln 50 55 60

Gly Ile Ser Val Ala Asp Glu Glu Asp Tyr Val Lys Ala Gly Gly Ser 65 70 75 80

Glu Leu Phe Phe Val Gln Met Gln Arg Thr Lys Ser Met Glu Ser Gln 85 90 95

Ser Lys Leu Ser Glu Lys Leu Ala Gln Ile Pro Ile Gly Asn Cys Ile 100 105 110

Leu Asp Leu Val Val Ile Gly Cys Gly Pro Ala Gly Leu Ala Leu Ala 115 120 125

Ala Glu Ser Ala Lys Leu Gly Leu Asn Val Gly Leu Ile Gly Pro Asp 130 135 140

Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Gln Asp Glu Phe Ile Gly 145 150 155 160

Leu Gly Leu Glu Gly Cys Ile Glu His Ser Trp Lys Asp Thr Leu Val 165 170 175

Tyr Leu Asp Asp Ala Asp Pro Ile Arg Ile Gly Arg Ala Tyr Gly Arg 180 185 190

Val His Arg Asp Leu Leu His Glu Glu Leu Leu Arg Arg Cys Val Glu 195 200 205

Ser Gly Val Ser Tyr Leu Ser Ser Lys Val Glu Arg Ile Thr Glu Ala 210 215 220

Pro Asn Gly Tyr Ser Leu Ile Glu Cys Glu Gly Asn Ile Thr Ile Pro

			•		7										
225					230					235					240
Cys	Arg	Leu	Ala	Thr 245	Val	Ala	Ser	Gly	Ala 250	Ala	Ser	Gly	Lys	Phe 255	Leu
Glu	Tyr	Glu	Leu 260	Gly	Gly	Pro	Arg	Val 265	Cys	Val	Gln	Thr	Ala 270	Tyr	Gly
Ile	Glu	Val 275	Glu	Val	Glu	Asn	Asn 280	Pro	Tyr	Asp	Pro	Asp 285	Leu	Met	Val
Phe	Met 290	Asp	Tyr	Arg	Asp	Phe 295	Ser	Lys	His	Lys	Pro 300	Glu	Ser	Leu	Glu
Ala 305	Lys	Tyr	Pro	Thr	Phe 310	Leu	Tyr	Val	Met	Ala 315	Met	Ser	Pro	Thr	Lys 320
Ile	Phe	Phe	Glu	Glu 325	Thr	Cys	Leu	Ala	Ser 330	Arg	Glu	Ala	Met	Pro 335	Phe
Asn	Leu	Leu	Lys 340	Ser	Lys	Leu	Met	Ser 345	Arg	Leu	Lys	Ala	Met 350	Gly	Ile
Arg	Ile	Thr 355	Arg	Thr	Tyr	Glu	Glu 360	Glu	Trp	Ser	Tyr	Ile 365	Pro	Val	Gly
Gly	Ser 370	Leu	Pro	Asn	Thr	Glu 375	Gln	Lys	Asn	Leu	Ala 380	Phe	Gly	Ala	Ala
Ala 385	Ser	Met	Val	His	Pro 390	Ala	Thr	Gly	Tyr	Ser 395	Val	Val	Arg	Ser	Leu 400
Ser	Glu	Ala	Pro	Asn 405	Tyr	Ala	Ala	Val	Ile 410	Ala	Lys	Ile	Leu	Arg 415	Gln
Asp	Gln	Ser	Lys 420	Glu	Met	Ile	Ser	Leu 425	Gly	Lys	Tyr	Thr	Asn 430	Ile	Ser
Lys	Gln	Ala 435	Trp	Glu	Thr	Leu	Trp 440	Pro	Leu	Glu	Arg	Lys 445	Arg	Gln	Arg
Ala	Phe 450	Phe	Leu	Phe	Gly	Leu 455	Ser	His	Ile	Val	Leu 460	Met	Asp	Leu	Glu
Gly 465	Thr	Arg	Thr	Phe	Phe 470	Arg	Thr	Phe	Phe	Arg 475	Leu	Pro	Lys	Trp	Met 480
Trp	Trp	Gly	Phe	Leu 485	Gly	Ser	Ser	Leu	Ser 490	Ser	Thr	Asp	Leu	Ile 495	Ile
Phe	Ala	Leu	Tyr 500	Met	Phe	Val	Ile	Ala 505	Pro	His	Ser	Leu	Arg 510	Met	Glu
Leu	Val	Arg 515	His	Leu	Leu.	Ser	Asp 520	Pro	Thr	Gly	Ala	Thr 525	Met	Val	Lys
Ala	Tyr 530	Leu	Thr	Ile											

<210> 53

<211> 526 <212> PRT

<213> Tomato

<400> 53

Met Glu Cys Val Gly Val Gln Asn Val Gly Ala Met Ala Val Leu Thr 1 5 10 15

Arg Pro Arg Leu Asn Arg Trp Ser Gly Gly Glu Leu Cys Gln Glu Lys 20 25 30

Ser Ile Phe Leu Ala Tyr Glu Gln Tyr Glu Ser Lys Cys Asn Ser Ser 35 40 45

Ser Gly Ser Asp Ser Cys Val Val Asp Lys Glu Asp Phe Ala Asp Glu 50 55 60

Glu Asp Tyr Ile Lys Ala Gly Gly Ser Gln Leu Val Phe Val Gln Met 65 70 75 80

Gln Gln Lys Lys Asp Met Asp Gln Gln Ser Lys Leu Ser Asp Glu Leu 85 90 95

Arg Gln Ile Ser Ala Gly Gln Thr Val Leu Asp Leu Val Val Ile Gly
100 105 110

Cys Gly Pro Ala Gly Leu Ala Leu Ala Ala Glu Ser Ala Lys Leu Gly 115 120 125

Leu Asn Val Gly Leu Val Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr 130 135 140

Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Gln Ala Cys Ile 145 150 155 160

Glu His Val Trp Arg Asp Thr Ile Val Tyr Leu Asp Asp Glu Pro 165 170 175

Ile Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Phe Leu His 180 185 190

Glu Glu Leu Leu Lys Arg Cys Val Glu Ala Gly Val Leu Tyr Leu Asn 195 200 205

Ser Lys Val Asp Arg Ile Val Glu Ala Thr Asn Gly Gln Ser Leu Val 210 215 220

Glu Cys Glu Gly Asp Val Val Ile Pro Cys Arg Phe Val Thr Val Ala 225 230 235 240

Ser Gly Ala Ala Ser Gly Lys Phe Leu Gln Tyr Glu Leu Gly Ser Pro 245 250 255

Arg Val Ser Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Asp Asn 260 265 270

Asn Pro Phe Asp Pro Ser Leu Met Val Phe Met Asp Tyr Arg Asp Tyr 275 280 285

Leu Arg His Asp Ala Gln Ser Leu Glu Ala Lys Tyr Pro Thr Phe Leu 290 295 300

Tyr Ala Met Pro Met Ser Pro Thr Arg Val Phe Phe Glu Glu Thr Cys 305 310 315 Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Lys Leu Met Leu Arg Leu Asn Thr Leu Gly Val Arg Ile Lys Glu Ile Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln Lys Thr Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys Cys Ala Ser Val Leu Ala Asn Ile Leu Arg Gln His Tyr Ser Lys Asn Met Leu 410 Thr Ser Ser Ser Ile Pro Ser Ile Ser Thr Gln Ala Trp Asn Thr Leu Trp Pro Gln Glu Arg Lys Arg Gln Arg Ser Phe Phe Leu Phe Gly Leu 440 Ala Leu Ile Leu Gln Leu Asp Ile Glu Gly Ile Arg Ser Phe Phe Arg 455 Ala Phe Phe Arg Val Pro Lys Trp Met Trp Gln Gly Phe Leu Gly Ser Ser Leu Ser Ser Ala Asp Leu Met Leu Phe Ala Phe Tyr Met Phe Ile Ile Ala Pro Asn Asp Met Arg Lys Gly Leu Ile Arg His Leu Leu Ser 505 Asp Pro Thr Gly Ala Thr Leu Ile Arg Thr Tyr Leu Thr Phe 515

<210> 54

<211> 516

<212> PRT

<213> Tagetes erecta

<400> 54

Met Ser Met Arg Ala Gly His Met Thr Ala Thr Met Ala Ala Phe Thr 1 5 10 15

Cys Pro Arg Phe Met Thr Ser Ile Arg Tyr Thr Lys Gln Ile Lys Cys 20 25 30

Asn Ala Ala Lys Ser Gln Leu Val Val Lys Gln Glu Ile Glu Glu Glu 35 40 45

Glu Asp Tyr Val Lys Ala Gly Gly Ser Glu Leu Leu Phe Val Gln Met 50 55 60



Gln 65	Gln	Asn	Lys	Ser	Met 70	Asp	Ala	Gln	Ser	Ser 75	Leu	Ser	Gln	Lys	Leu 80
Pro	Arg	Val	Pro	Ile 85	Gly	Gly	Gly	Gly	Asp 90	Ser	Asn	Cys	Ile	Leu 95	Asp
Leu	Val	Val	Ile 100	Gly	Cys	Gly	Pro	Ala 105	Gly	Leu	Ala	Leu	Ala 110	Gly	Glu
Ser	Ala	Lys 115	Leu	Gly	Leu	Asn	Val 120	Ala	Leu	Ile	Gly	Pro 125	Asp	Leu	Pro
Phe	Thr 130	Așn	Asn	Tyr	Gly	Val 135	Trp	Glu	Asp	Glu	Phe 140	Ile	Gly	Leu	Gly
Leu 145	Glu	Gly	Cys	Ile	Glu 150	His	Val	Trp	Arg	Asp 155	Thr	Val	Val	Tyr	Leu 160
Asp	Asp	Asn	Asp	Pro 165	Ile	Leu	Ile	Gly	Arg 170	Ala	Tyr	Gly	Arg	Val 175	Ser
Arg	Asp	Leu	Leu 180	His	Glu	Glu	Leu	Leu 185	Thr	Arg	Cys	Met	Glu 190	Ser	Gly
Val	Ser	Tyr 195	Leu	Ser	Ser	Lys	Val 200	Glu	Arg	Ile	Thr	Glu 205	Ala	Pro	Asn
Gly	Leu 210	Ser	Leu	Ile	Glu	Cys 215	Glu	Gly	Asn	Ile	Thr 220	Ile	Pro	Cys	Arg
Leu 225	Ala	Thr	Val	Ala	Ser 230	Gly	Ala	Ala	Ser	Gly 235	Lys	Leu	Leu	Gln	Tyr 240
Glu	Leu	Gly	Gly	Pro 2 <b>4</b> 5	Arg	Val	Cys	Val	Gln 250	Thr	Ala	Tyr	Gly	Ile 255	Glu
Val	Glu	Val	Glu 260	Ser	Ile	Pro	Tyr	Asp 265	Pro	Ser	Leu	Met	Val 270	Phe	Met
Asp	Tyr	Arg 275	Asp	Tyr	Thr	Lys	His 280	Lys	Ser	Gln	Ser	Leu 285	Glu	Ala	Gln
Tyr	Pro 290	Thr	Phe	Leu	Tyr	Val 295	Met	Pro	Met	Ser	Pro 300	Thr	Lys	Val	Phe
Phe 305	Glu	Glu	Thr	Cys	Leu 310	Ala	Ser	Lys	Glu	Ala 315	Met	Pro	Phe	Glu	Leu 320
Leu	Lys	Thr	Lys	Leu 325	Met	Ser	Arg	Leu	Lys 330	Thr	Met	Gly	Ile	Arg 335	Ile
Thr	Lys	Thr	Tyr 340	Glu	Glu	Glu	Trp	Ser 345	Tyr	Ile	Pro	Val	Gly 350	Gly	Ser
Leu	Pro	Asn 355	Thr	Glu	Gln	Lys	Asn 360	Leu	Ala	Phe	Gly	Ala 365	Ala	Ala	Ser
Met	Val 370	His	Pro	Ala	Thr	Gly 375	Tyr	Ser	Val	Val	Arg 380	Ser	Leu	Ser	Glu
Ala	Pro	Asn	Tyr	Ala	Ala	Val	Ile	Ala	Lys	Ile	Leu	Gly	Lys	Gly	Asn

PCT/US99/12121

WO 99/63055 390 385 395 Ser Lys Gln Met Leu Asp His Gly Arg Tyr Thr Thr Asn Ile Ser Lys Gln Ala Trp Glu Thr Leu Trp Pro Leu Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu Phe Gly Leu Ala Leu Ile Val Gln Met Asp Ile Glu Gly 440 Thr Arg Thr Phe Phe Arg Thr Phe Phe Arg Leu Pro Thr Trp Met Trp Trp Gly Phe Leu Gly Ser Ser Leu Ser Ser Thr Asp Leu Ile Ile Phe Ala Phe Tyr Met Phe Ile Ile Ala Pro His Ser Leu Arg Met Gly Leu Val Arg His Leu Leu Ser Asp Pro Thr Gly Gly Thr Met Leu Lys Ala Tyr Leu Thr Ile 515 <210> 55 <211> 501 <212> PRT <213> Arabidopsis thaliana <400> 55

Met Asp Thr Leu Leu Lys Thr Pro Asn Lys Leu Asp Phe Phe Ile Pro

Gln Phe His Gly Phe Glu Arg Leu Cys Ser Asn Asn Pro Tyr His Ser

Arg Val Arg Leu Gly Val Lys Lys Arg Ala Ile Lys Ile Val Ser Ser

Val Val Ser Gly Ser Ala Ala Leu Leu Asp Leu Val Pro Glu Thr Lys

Lys Glu Asn Leu Asp Phe Glu Leu Pro Leu Tyr Asp Thr Ser Lys Ser

Gln Val Val Asp Leu Ala Ile Val Gly Gly Pro Ala Gly Leu Ala

Val Ala Gln Gln Val Ser Glu Ala Gly Leu Ser Val Cys Ser Ile Asp

Pro Ser Pro Lys Leu Ile Trp Pro Asn Asn Tyr Gly Val Trp Val Asp

Glu Phe Glu Ala Met Asp Leu Leu Asp Cys Leu Asp Thr Thr Trp Ser 135

Gly Ala Val Val Tyr Val Asp Glu Gly Val Lys Lys Asp Leu Ser Arg



145					150					155					160
Pro	Tyr	Gly	Arg	Val 165	Asn	Arg	Lys	Gln	Leu 170	Lys	Ser	Lys	Met	Leu 175	Gln
Lys	Cys	Ile	Thr 180	Asn	Gly	Val	Lys	Phe 185	His	Gln	Ser	Lys	Val 190	Thr	Asn
Val	Val	His 195	Glu	Glu	Ala	Asn	Ser 200	Thr	Val	Val	Cys	Ser 205	Asp	Gly	Val
Lys	Ile 210	Gln	Ala	Ser	Val	Val 215	Leu	Asp	Ala	Thr	Gly 220	Phe	Ser	Arg	Cys
Leu 225	Val	Gln	Tyr	Asp	Lys 230	Pro	Tyr	Asn	Pro	Gly 235	Tyr	Gln	Val	Ala	Tyr 240
Gly	Ile	Val	Ala	Glu 245	Val	Asp	Gly	His	Pro 250	Phe	Asp	Val	Asp	Lys 255	Met
Val	Phe	Met	Asp 260	Trp	Arg	Asp	Lys	His 265	Leu	Asp	Ser	Tyr	Pro 270	Glu	Leu
Lys	Glu	Arg 275	Asn	Ser	Lys	Ile	Pro 280	Thr	Phe	Leu	Tyr	Ala 285	Met	Pro	Phe
Ser	Ser 290	Asn	Arg	Ile	Phe	Leu 295	Glu	Glu	Thr	Ser	Leu 300	Val	Ala	Arg	Pro
Gly 305	Leu	Arg	Met	Glu	Asp 310	Ile	Gln	Glu	Arg	Met 315	Ala	Ala	Arg	Leu	Lys 320
His	Leu	Gly	Ile	Asn 325	Val	Lys	Arg	Ile	Glu 330	Glu	Asp	Glu	Arg	Cys 335	Val
Ile	Pro	Met	Gly 340	Gly	Pro	Leu	Pro	Val 345	Leu	Pro	Gln	Arg	Val 350	Val	Gly
Ile	Gly	Gly 355	Thr	Ala	Gly	Met	Val 360	His	Pro	Ser	Thr	Gly 365	Tyr	Met	Val
Ala	Arg 370	Thr	Leu	Ala	Ala	Ala 375	Pro	Ile	Val	Ala	Asn 380	Ala	Ile	Val	Arg
Tyr 385	Leu	Gly	Ser	Pro	Ser 390	Ser	Asn	Ser	Leu	Arg 395	Gly	Asp	Gln	Leu	Ser 400
Ala	Glu	Val	Trp	Arg 405	Asp	Leu	Trp	Pro	Ile 410	Glu	Arg	Arg	Arg	Gln 415	Arg
Glu	Phe	Phe	Cys 420	Phe	Gly	Met	Asp	Ile 425	Leu	Leu	Lys	Leu	Asp 430	Leu	Asp
Ala	Thr	Arg 435	Arg	Phe	Phe	Asp	Ala 440	Phe	Phe	Asp	Leu	Gln 445	Pro	His	Tyr
Trp	His 450	Gly	Phe	Leu	Ser	Ser 455	Arg	Leu	Phe	Leu	Pro 460	Glu	Leu	Leu	Val
Phe 465	Gly	Leu	Ser	Leu	Phe 470	Ser	His	Ala	Ser	Asn 475	Thr	Ser	Arg	Leu	Glu 480



Ile Met Thr Lys Gly Thr Val Pro Leu Ala Lys Met Ile Asn Asn Leu 485 490 495

Val Gln Asp Arg Asp 500

<210> 56

<211> 502

<212> PRT

<213> Adonis palaestina

<400> 56

Met Asp Thr Leu Leu Arg Thr His Asn Lys Leu Glu Leu Leu Pro Thr 1 5 10 15

Leu His Gly Phe Ala Glu Lys Gln His Leu Val Ser Thr Ser Lys Leu 20 25 30

Gln Asn Gln Val Phe Arg Ile Ala Ser Arg Asn Ile His Pro Cys Arg 35 40 45

Asn Gly Thr Val Lys Ala Arg Gly Ser Ala Leu Leu Glu Leu Val Pro 50 60

Glu Thr Lys Lys Glu Asn Leu Glu Phe Asp Leu Pro Ala Tyr Asp Pro 65 70 75 80

Ser Arg Gly Ile Val Val Asp Leu Ala Val Val Gly Gly Pro Ala 85 90 95

Gly Leu Ala Ile Ala Gln Gln Val Ser Glu Ala Gly Leu Leu Val Cys 100 105 110

Ser Ile Asp Pro Ser Pro Lys Leu Ile Trp Pro Asn Asn Tyr Gly Val 115 120 125

Trp Val Asp Glu Phe Glu Ala Met Asp Leu Leu Asp Cys Leu Asp Thr 130 135 140

Thr Trp Ser Gly Ala Val Val Tyr Thr Asp Asp Asn Ser Lys Lys Tyr 145 150 155 160

Leu Asp Arg Pro Tyr Gly Arg Val Asn Arg Lys Gln Leu Lys Ser Lys 165 170 175

Met Leu Gln Lys Cys Val Thr Asn Gly Val Lys Phe His Gln Ala Lys 180 185 190

Val Ile Lys Val Ile His Glu Glu Ser Lys Ser Leu Leu Ile Cys Asn 195 200 205

Asp Gly Ile Thr Ile Asn Ala Thr Val Val Leu Asp Ala Thr Gly Phe 210 215 220

Ser Arg Cys Leu Val Gln Tyr Asp Lys Pro Tyr Asn Pro Gly Tyr Gln 225 230 235 240

Val Ala Tyr Gly Ile Met Ala Glu Val Glu Glu His Pro Phe Asp Leu 245 250 255



Asp	Lys	Met	Leu 260	Phe	Met	Asp	Trp	Arg 265	Asp	Ser	His	Leu	Asn 270	Glu	Lys
Leu	Glu	Leu 275	Lys	Asp	Lys	Asn	Arg 280	Lys	Ile	Pro	Thr	Phe 285	Leu	Tyr	Ala
Met	Pro 290	Phe	Ser	Ser	Thr	Lys 295	Ile	Phe	Leu	Glu	Glu 300	Thr	Ser	Leu	Val
Ala 305	Arg	Pro	Gly	Leu	Arg 310	Phe	Glu	Asp	Ile	Gln 315	Glu	Arg	Met	Val	Ala 320
Arg	Leu	Lys	His	Leu 325	Gly	Ile	Lys	Val	Lys 330	Ser	Ile	Glu	Glu	Asp 335	Glu
Arg	Cys	Val	Ile 340	Pro	Met	Gly	Gly	Pro 345	Leu	Pro	Val	Leu	Pro 350	Gln	Arg
Val	Val	Gly 355	Ile	Gly	Gly	Thr	Ala 360	Gly	Met	Val	His	Pro 365	Ser	Thr	Gly
Tyr	Met 370	Val	Ala	Arg	Thr	Leu 375	Ala	Ala	Ala	Pro	Val 380	Val	Ala	Lys	Ser
Ile 385	Val	Gln	Tyr	Leu	Gly 390	Ser	Asp	Arg	Ser	Leu 395	Ser	Gly	Asn	Glu	Leu 400
Ser	Ala	Glu	Val	Trp 405	Lys	Asp	Leu	Trp	Pro 410	Ile	Glu	Arg	Arg	Arg 415	Gln
Arg	Glu	Phe	Phe 420	Cys	Phe	Gly	Met	Asp 425	Ile	Leu	Leu	Lys	Leu 430	Asp	Leu
Gln	Gly	Thr 435	Arg	Arg	Phe	Phe	Asp 440	Ala	Phe	Phe	Asp	Leu 445	Glu	Pro	His
Tyr	Trp 450	His	Gly	Phe	Leu	Ser 455	Ser	Arg	Leu	Phe	Leu 460	Pro	Glu	Leu	Leu
Phe 465	Phe	Gly	Leu	Ser	Leu 470	Phe	Ser	His	Ala	Ser 475	Asn	Ala	Ser	Arg	Ile 480
Glu	Ile	Met	Ala	Lys 485	Gly	Thr	Val	Pro	Leu 490	Val	Asn	Met	Met	Asn 495	Asn
Leu	Ile	Gln	Asp 500	Thr	Asp										

500

<210> 57 <211> 498 <212> PRT <213> Pepper

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Phe Gly Val Lys Val Ser Ala Phe Ser Ser Val Lys Ser Gln Lys Phe 20



Gly Ala Lys Lys Phe Cys Glu Gly Leu Gly Ser Arg Ser Val Cys Val Lys Ala Ser Ser Ser Ala Leu Leu Glu Leu Val Pro Glu Thr Lys Lys Glu Asn Leu Asp Phe Glu Leu Pro Met Tyr Asp Pro Ser Lys Gly Val Val Val Asp Leu Ala Val Val Gly Gly Pro Ala Gly Leu Ala Val Ala Gln Gln Val Ser Glu Ala Gly Leu Ser Val Cys Ser Ile Asp Pro Asn Pro Lys Leu Ile Trp Pro Asn Asn Tyr Gly Val Trp Val Asp Glu Phe Glu Ala Met Asp Leu Leu Asp Cys Leu Asp Ala Thr Trp Ser Gly Ala Ala Val Tyr Ile Asp Asp Lys Thr Thr Lys Asp Leu Asn Arg Pro 150 Tyr Gly Arg Val Asn Arg Lys Gln Leu Lys Ser Lys Met Met Gln Lys Cys Ile Leu Asn Gly Val Lys Phe His Gln Ala Lys Val Ile Lys Val Ile His Glu Glu Ser Lys Ser Met Leu Ile Cys Asn Asp Gly Ile Thr 200 Ile Gln Ala Thr Val Val Leu Asp Ala Thr Gly Phe Ser Arg Ser Leu 210 Val Gln Tyr Asp Lys Pro Tyr Asn Pro Gly Tyr Gln Val Ala Tyr Gly Ile Leu Ala Glu Val Glu Glu His Pro Phe Asp Val Asn Lys Met Val Phe Met Asp Trp Arg Asp Ser His Leu Lys Asn Asn Val Glu Leu Lys 265 Glu Arg Asn Ser Arg Ile Pro Thr Phe Leu Tyr Ala Met Pro Phe Ser Ser Asn Arg Ile Phe Leu Glu Glu Thr Ser Leu Val Ala Arg Pro Gly 295 Leu Gly Met Asp Asp Ile Gln Glu Arg Met Val Ala Arg Leu Ser His Leu Gly Ile Lys Val Lys Ser Ile Glu Glu Asp Glu His Cys Val Ile Pro Met Gly Gly Pro Leu Pro Val Leu Pro Gln Arg Val Val Gly Ile 345 Gly Gly Thr Ala Gly Met Val His Pro Ser Thr Gly Tyr Met Val Ala



355 360 365

Arg Thr Leu Ala Ala Ala Pro Val Val Ala Asn Ala Ile Ile Gln Tyr 370 380

Leu Ser Ser Glu Arg Ser His Ser Gly Asp Glu Leu Ser Ala Ala Val 385 390 395 400

Trp Lys Asp Leu Trp Pro Ile Glu Arg Arg Arg Gln Arg Glu Phe Phe
405 410 415

Cys Phe Gly Met Asp Ile Leu Leu Lys Leu Asp Leu Pro Ala Thr Arg 420 425 430

Arg Phe Phe Asp Ala Phe Phe Asp Leu Glu Pro Arg Tyr Trp His Gly 435 440 445

Phe Leu Ser Ser Arg Leu Phe Leu Pro Glu Leu Ile Val Phe Gly Leu 450 455 460

Ser Leu Phe Ser His Ala Ser Asn Thr Ser Arg Leu Glu Ile Met Thr 465 470 475 480

Lys Gly Thr Leu Pro Leu Val His Met Ile Asn Asn Leu Leu Gln Asp 485 490 495

Lys Glu

<210> 58

<211> 500

<212> PRT

<213> Tomato

<400> 58

Met Asp Thr Leu Leu Lys Thr Pro Asn Asn Leu Glu Phe Leu Asn Pro 1 5 10 15

His His Gly Phe Ala Val Lys Ala Ser Thr Phe Arg Ser Glu Lys His 20 25 30

His Asn Phe Gly Ser Arg Lys Phe Cys Glu Thr Leu Gly Arg Ser Val 35 40 45

Cys Val Lys Gly Ser Ser Ser Ala Leu Leu Glu Leu Val Pro Glu Thr 50 55 60

Lys Lys Glu Asn Leu Asp Phe Glu Leu Pro Met Tyr Asp Pro Ser Lys 65 70 75 80

Gly Val Val Asp Leu Ala Val Val Gly Gly Pro Ala Gly Leu 85 90 95

Ala Val Ala Gln Gln Val Ser Glu Ala Gly Leu Ser Val Cys Ser Ile 100 105 110

Asp Pro Asn Pro Lys Leu Ile Trp Pro Asn Asn Tyr Gly Val Trp Val 115 120 125

Asp Glu Phe Glu Ala Met Asp Leu Leu Asp Cys Leu Asp Ala Thr Trp



130 135 140

Ser Gly Ala Ala Val Tyr Ile Asp Asp Asn Thr Ala Lys Asp Leu His 155 Arg Pro Tyr Gly Arg Val Asn Arg Lys Gln Leu Lys Ser Lys Met Met Gln Lys Cys Ile Met Asn Gly Val Lys Phe His Gln Ala Lys Val Ile Lys Val Ile His Glu Glu Ser Lys Ser Met Leu Ile Cys Asn Asp Gly Ile Thr Ile Gln Ala Thr Val Val Leu Asp Ala Thr Gly Phe Ser Arg Ser Leu Val Gln Tyr Asp Lys Pro Tyr Asn Pro Gly Tyr Gln Val Ala Tyr Gly Ile Leu Ala Glu Val Glu Glu His Pro Phe Asp Val Asn Lys Met Val Phe Met Asp Trp Arg Asp Ser His Leu Lys Asn Asn Thr Asp 265 Leu Lys Glu Arg Asn Ser Arg Ile Pro Thr Phe Leu Tyr Ala Met Pro 280 Phe Ser Ser Asn Arg Ile Phe Leu Glu Glu Thr Ser Leu Val Ala Arg Pro Gly Leu Arg Ile Asp Asp Ile Gln Glu Arg Met Val Ala Arg Leu Asn His Leu Gly Ile Lys Val Lys Ser Ile Glu Glu Asp Glu His Cys Leu Ile Pro Met Gly Gly Pro Leu Pro Val Leu Pro Gln Arg Val Val 345 Gly Ile Gly Gly Thr Ala Gly Met Val His Pro Ser Thr Gly Tyr Met Val Ala Arg Thr Leu Ala Ala Pro Val Val Ala Asn Ala Ile Ile 375 380 Gln Tyr Leu Gly Ser Glu Arg Ser His Ser Gly Asn Glu Leu Ser Thr Ala Val Trp Lys Asp Leu Trp Pro Ile Glu Arg Arg Gln Arg Glu 410 Phe Phe Cys Phe Gly Met Asp Ile Leu Leu Lys Leu Asp Leu Pro Ala Thr Arg Arg Phe Phe Asp Ala Phe Phe Asp Leu Glu Pro Arg Tyr Trp His Gly Phe Leu Ser Ser Arg Leu Phe Leu Pro Glu Leu Ile Val Phe 455

Gly Leu Ser Leu Phe Ser His Ala Ser Asn Thr Ser Arg Phe Glu Ile 465 470 475 480

Met Thr Lys Gly Thr Val Pro Leu Val Asn Met Ile Asn Asn Leu Leu 485 490 495

Gln Asp Lys Glu 500

<210> 59

<211> 500

<212> PRT

<213> Tobacco

<400> 59

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Val His Gly Phe Ser Val Lys Ala Ser Ser Phe Asn Ser Val Lys Pro 20 25 30

His Lys Phe Gly Ser Arg Lys Ile Cys Glu Asn Trp Gly Lys Gly Val 35 40 45

Cys Val Lys Ala Lys Ser Ser Ala Leu Leu Glu Leu Val Pro Glu Thr 50 55 60

Lys Lys Glu Asn Leu Asp Phe Glu Leu Pro Met Tyr Asp Pro Ser Lys 65 70 75 80

Gly Leu Val Val Asp Leu Ala Val Val Gly Gly Pro Ala Gly Leu 85 90 95

Ala Val Ala Gl<br/>n Gl<br/>n Val Ser Glu Ala Gly Leu Ser Val Val Ser Ile 100 \$105<br/> 110

Asp Pro Ser Pro Lys Leu Ile Trp Pro Asn Asn Tyr Gly Val Trp Val 115 120 125

Asp Glu Phe Glu Ala Met Asp Leu Leu Asp Cys Leu Asp Ala Thr Trp 130 135 140

Ser Gly Thr Val Val Tyr Ile Asp Asp Asn Thr Thr Lys Asp Leu Asp 145 150 155 160

Arg Pro Tyr Gly Arg Val Asn Arg Lys Gln Leu Lys Ser Lys Met Met 165 170 175

Gln Lys Cys Ile Leu Asn Gly Val Lys Phe His His Ala Lys Val Ile 180 180

Lys Val Ile His Glu Glu Ala Lys Ser Met Leu Ile Cys Asn Asp Gly
195 200 205

Val Thr Ile Gln Ala Thr Val Val Leu Asp Ala Thr Gly Phe Ser Arg 210 215 220

Cys Leu Val Gln Tyr Asp Lys Pro Tyr Lys Pro Gly Tyr Gln Val Ala 225 230 235 240



Tyr Gly Ile Leu Ala Glu Val Glu Glu His Pro Phe Asp Thr Ser Lys 245 250 Met Val Leu Met Asp Trp Arg Asp Ser His Leu Gly Asn Asn Met Glu Leu Lys Glu Arg Asn Arg Lys Val Pro Thr Phe Leu Tyr Ala Met Pro Phe Ser Ser Asn Lys Ile Phe Leu Glu Glu Thr Ser Leu Val Ala Arg 295 Pro Gly Leu Arg Met Asp Asp Ile Gln Glu Arg Met Val Ala Arg Leu Asn His Leu Gly Ile Lys Val Lys Ser Ile Glu Glu Asp Glu His Cys Val Ile Pro Met Gly Gly Ser Leu Pro Val Ile Pro Gln Arg Val Val Gly Thr Gly Gly Thr Ala Gly Leu Val His Pro Ser Thr Gly Tyr Met Val Ala Arg Thr Leu Ala Ala Ala Pro Val Val Ala Asn Ala Ile Ile 375 His Tyr Leu Gly Ser Glu Lys Asp Leu Leu Gly Asn Glu Leu Ser Ala 390 395 Ala Val Trp Lys Asp Leu Trp Pro Ile Glu Arg Arg Arg Gln Arg Glu Phe Phe Cys Phe Gly Met Asp Ile Leu Leu Lys Leu Asp Leu Pro Ala Thr Arg Arg Phe Phe Asp Ala Phe Phe Asp Leu Glu Pro Arg Tyr Trp 440 His Gly Phe Leu Ser Ser Arg Leu Tyr Leu Pro Glu Leu Ile Phe Phe Gly Leu Ser Leu Phe Ser Arg Ala Ser Asn Thr Ser Arg Ile Glu Ile 470 475 Met Thr Lys Gly Thr Leu Pro Leu Val Asn Met Ile Asn Asn Leu Leu

490

Gln Asp Thr Glu

<210> 60

<211> 511

<212> PRT

<213> Tagetes erecta

<400> 60

Met Asp Thr Phe Leu Arg Thr Tyr Asn Ser Phe Glu Phe Val His Pro

Ser Asn Lys Phe Ala Gly Asn Leu Asn Asn Leu Asn Gln Leu Asn Gln Ser Lys Ser Gln Phe Gln Asp Phe Arg Phe Gly Pro Lys Lys Ser Gln Phe Lys Leu Gly Gln Lys Tyr Cys Val Lys Ala Ser Ser Ser Ala Leu Leu Glu Leu Val Pro Glu Ile Lys Lys Glu Asn Leu Asp Phe Asp Leu Pro Met Tyr Asp Pro Ser Arg Asn Val Val Val Asp Leu Val Val Val Gly Gly Gro Ser Gly Leu Ala Val Ala Gln Gln Val Ser Glu Ala Gly Leu Thr Val Cys Ser Ile Asp Pro Ser Pro Lys Leu Ile Trp Pro Asn Asn Tyr Gly Val Trp Val Asp Glu Phe Glu Ala Met Asp Leu Leu Asp Cys Leu Asp Thr Thr Trp Ser Ser Ala Val Val Tyr Ile Asp Glu 150 155 Lys Ser Thr Lys Ser Leu Asn Arg Pro Tyr Ala Arg Val Asn Arg Lys 165 Gln Leu Lys Thr Lys Met Leu Gln Lys Cys Ile Ala Asn Gly Val Lys Phe His Gln Ala Lys Val Ile Lys Val Ile His Glu Glu Leu Lys Ser Leu Leu Ile Cys Asn Asp Gly Val Thr Ile Gln Ala Thr Leu Val Leu 215 Asp Ala Thr Gly Phe Ser Arg Ser Leu Val Gln Tyr Asp Lys Pro Tyr Asn Pro Gly Tyr Gln Val Ala Tyr Gly Ile Leu Ala Glu Val Glu Glu 245 250 His Pro Phe Asp Val Asp Lys Met Leu Phe Met Asp Trp Arg Asp Ser 265 His Leu Asp Gln Asn Leu Glu Ile Lys Ala Arg Asn Ser Arg Ile Pro 280 Thr Phe Leu Tyr Ala Met Pro Phe Ser Ser Thr Arg Ile Phe Leu Glu 295 Glu Thr Ser Leu Val Ala Arg Pro Gly Leu Lys Met Glu Asp Ile Gln Glu Arg Met Ala Tyr Arg Leu Lys His Leu Gly Ile Lys Val Lys Ser Ile Glu Glu Asp Glu Arg Cys Val Ile Pro Met Gly Gly Pro Leu Pro



340 345 350

Val Leu Pro Gln Arg Val Leu Gly Ile Gly Gly Thr Ala Gly Met Val 355 Gln Arg Val Leu Gly Ile Gly Gly Thr Ala Gly Met Val 365 Gly Reights From Ser Thr Gly Tyr Met Val Ala Arg Thr Leu Ala Ala Ala Pro 370 Ser Thr Gly Tyr Met Val Ala Arg Thr Leu Ala Ala Ala Ala Pro 385 Val Ala Lys Ser Ile Ile Arg Tyr Leu Ala Ala Gly Lys Ser Met 400 Val Ala Asp Val Thr Gly Asp Asp Leu Ala Ala Gly Ile Trp Arg Glu 415 Gly Ile Trp Arg Glu Ala Ala Ser Ala Reights From Arg Ile Leu Leu Lys Leu Ala Ala Gly Ile Trp Arg Arg Phe Phe 435 Pro Arg Tyr Trp His Gly Phe Leu Ser 445 Arg Leu Phe 445 Arg Leu Pro Ala Ser Ala Reights From Arg Val Gly Ile Met Ala Lys Gly Thr Ala Ser

<210> 61 <211> 503

<212> PRT

<213> Daffodil

<400> 61

Met Asp Thr Leu Leu Arg Thr His Asn Arg Leu Glu Leu Leu Tyr Pro 1 5 10 15

Leu His Glu Leu Ala Lys Arg His Phe Leu Ser Pro Ser Pro Asn Pro 20 25 30

Gln Asn Pro Asn Phe Lys Phe Phe Ser Arg Lys Pro Tyr Gln Lys Lys 35 40 45

Cys Arg Asn Gly Tyr Ile Gly Val Ser Ser Asn Gln Leu Leu Asp Leu
50 60

Val Pro Glu Ile Lys Lys Glu His Leu Glu Phe Asp Leu Pro Leu Tyr 65 70 75 80

Asp Pro Ser Lys Ala Leu Thr Leu Asp Leu Ala Val Gly Gly Gly 85 90 95

Pro Leu Ala Arg Ser Cys Ser Thr Ser Leu Gly Gly Gly Leu Ser Val  $100 \hspace{1cm} 105 \hspace{1cm} 110$ 

Val Ser Ile Asp Pro Asn Pro Lys Leu Ile Trp Pro Asn Asn Tyr Gly

115



120 125

		+					120					12,			
Val	Trp 130	Val	Asp	Glu	Phe	Glu 135	Asp	Met	Asp	Leu	Leu 140	Asp	Cys	Leu	Asp
Ala 145	Thr	Trp	Ser	Gly	Ala 150	Ile	Val	Tyr	Val	Asp 155	Asp	Arg	Ser	Thr	Lys 160
Asn	Leu	Ser	Arg	Pro 165	Tyr	Ala	Arg	Val	Asn 170	Arg	Lys	Asn	Leu	Lys 175	Ser
Lys	Met	Met	Lys 180	Lys	Cys	Val	Ser	Asn 185	Gly	Val	Arg	Phe	His 190	Gln	Ala
Thr	Val	Val 195	Lys	Ala	Met	His	Glu 200	Glu	Glu	Lys	Ser	Tyr 205	Leu	Ile	Cys
Ser	Asp 210	Gly	Val	Thr	Ile	Asp 215	Ala	Arg	Val	Val	Leu 220	Asp	Ala	Thr	Gly
Phe 225	Ser	Arg	Cys	Leu	Val 230	Gln	Tyr	Asp	Lys	Pro 235	Tyr	Asn	Pro	Gly	Tyr 240
Gln	Val	Ala	Tyr	Gly 245	Ile	Leu	Ala	Glu	Val 250	Glu	Glu	His	Pro	Phe 255	Asp
Val	Asp	Lys	Met 260	Val	Phe	Met	Asp	Trp 265	Arg	Asp	Ser	His	Leu 270	Asn	Gly
Lys	Ala	Glu 275	Leu	Asn	Glu	Arg	Asn 280	Ala	Lys	Ile	Pro	Thr 285	Phe	Leu	Tyr
Ala	Met 290	Pro	Phe	Ser	Ser	Asn 295	Arg	Ile	Phe	Leu	Glu 300	Glu	Thr	Ser	Leu
Val 305	Ala	Arg	Pro	Gly	Leu 310	Lys	Met	Glu	Asp	Ile 315	Gln	Glu	Arg	Met	Val 320
Ala	Arg	Leu	Asn	His 325	Leu	Gly	Ile	Arg	Ile 330	Lys	Ser	Ile	Glu	Glu 335	Asp
Glu	Arg	Cys	Val 340	Ile	Pro	Met	Gly	Gly 345	Pro	Leu	Pro	Val	Ile 350	Pro	Gln
Arg	Val	Val 355	Gly	Ile	Gly	Gly	Thr 360	Ala	Gly	Met	Val	His 365	Pro	Ser	Thr
Gly	Tyr 370	Met	Val	Ala	Arg	Thr 375	Leu	Ala	Ala	Ala	Pro 380	Ile	Val	Ala	Asn
Ser 385	Ile	Val	Gln	Tyr	Leu 390	Val	Ser	Asp	Ser	Gly 395	Leu	Ser	Gly	Asn	Asp 400
Leu	Ser	Ala	Asp	Val 405	Trp	Lys	Asp	Leu	Trp 410	Pro	Ile	Glu	Arg	Arg 415	Arg
Gln	Arg	Glu	Phe 420	Phe	Cys	Phe	Gly	Met 425	Asp	Ile	Leu	Leu	Lys 430	Leu	Asp
Leu	Glu	Gly 435	Thr	Arg	Arg	Phe	Phe 440	Asp	Ala	Phe	Phe	Asp 445	Leu	Glu	Pro



## WO 99/63055



Arg Tyr Trp His Gly Phe Leu Ser Ser Arg Leu Phe Leu Pro Glu Leu 450 460

Val Pro Phe Gly Leu Ser Leu Phe Ser His Ala Ser Asn Thr Cys Lys 465 470 475 480

Leu Glu Ile Met Ala Lys Gly Thr Leu Pro Leu Val Asn Met Ile Asn 485 490 495

Asn Leu Val Gln Asp Arg Asp 500



## INTERNATIONAL SEARCH REPORT

International application No. PCT/US99/12121

	SIFICATION OF SUBJECT MATTER lease See Extra Sheet.	
US CL :43	35/189, 193, 233, 252.3, 320.1, 325; 536/23.2 International Patent Classification (IPC) or to both r	national classification and IPC
B. FIELD	S SEARCHED	
Minimum doc	umentation searched (classification system followed	d by classification symbols)
U.S. : 43	5/189, 193, 233, 252.3, 320.1, 325; 536/23.2	
Documentation	n searched other than minimum documentation to the	e extent that such documents are included in the fields searched
Electronic data Please See I	·	ame of data base and, where practicable, search terms used)
C. DOCU	MENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where app	propriate, of the relevant passages Relevant to claim No.
	WO 97/36998 A1 (UNIVERSITY OF PARK) 09 October 1997, see entire d No:1.	
Further	r documents are listed in the continuation of Box C	See patent family annex.
• Speci	ial categories of cited documents:	*T* later document published after the international filing date or priority
	ment defining the general state of the art which is not considered of particular relevance	date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*E* earlie	er document published on or after the international filing date ment which may throw doubts on priority claim(s) or which is	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
cited	to establish the publication date of another citation or other all reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be
1 .	ment referring to an oral disclosure, use, exhibition or other	considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
the p	ment published prior to the international filing date but later than prority date claimed	*& document member of the same patent family
Date of the ac	ctual completion of the international search	Date of mailing of the international search report
02 AUGUS	T 1999	15 SEP 1999
Commissione Box PCT	ailing address of the ISA.US er of Patents and Trademarks	Authorized officer  BRADLEY S. MAYHEW  AUTHORITICAL STREET OF THE STREET
Washington, Facsimile No		Telephone No. (703) 368-0196
1	• •	1 · · ·



International application No. PCT/US99/12121

A. CLASSIFICATION OF SUBJECT MATTER: IPC (6):	
C12N 1/21, 5/10, 9/10, 15/53, 15/54, 15/61, 15/63; C12P 23/00; C12Q 1/68	
B. FIELDS SEARCHED Electronic data bases consulted (Name of data base and where practicable terms used):	
Dialog and APS search terms: IPP, epsilon cyclase, lycopene cyclase, isopentenyl pyrophosphate isomerase and isopentenyl diphosphate isomerase	